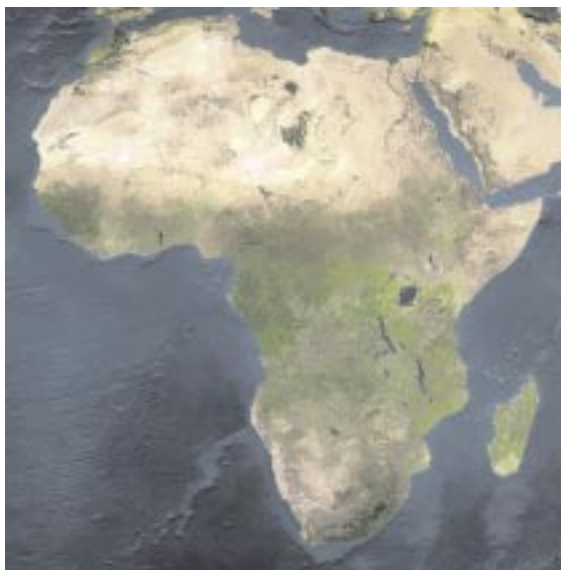
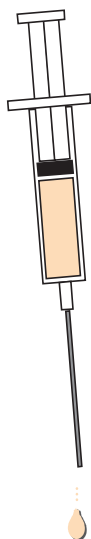




Live Vaccines for *Theileria parva*: Deployment in Eastern, Central and Southern Africa

Proceedings of an FAO/OAU–IBAR/ILRI Workshop
held at ILRI, Nairobi, Kenya
10–12 March 1997



ILRI Proceedings

International Livestock Research Institute

Live Vaccines for *Theileria parva*: Deployment in Eastern, Central and Southern Africa

Proceedings of an FAO/OAU–IBAR/ILRI Workshop
held at ILRI, Nairobi, Kenya
10–12 March 1997

Edited by
S. Morzaria and S. Williamson

Organising committee
A. Irvin, A. Musoke, S. Morzaria,
J. Mushiime and S. Mbogo



Organization of African Unity/Inter-African Bureau of Animal
Resources, P.O. Box 30786, Nairobi, Kenya



Food and Agriculture Organization of the United Nations
Via delle Terme di Caracalla, 00100 Rome, Italy



International Livestock Research Institute
P.O. Box 30709, Nairobi, Kenya

ISBN: 92-9146-055-9

Correct citation: Morzaria S. and Williamson S. (eds). 1999. *Live Vaccines for Theileria parva: Deployment in Eastern, Central and Southern Africa. Proceedings of an FAO, OAU-IBAR and ILRI Workshop held at ILRI, Nairobi, Kenya 10-12 March 1997*. ILRI (International Livestock Research Institute) Nairobi, Kenya. 166 pp.

Contents

Preface	
<i>Subhash Morzaria</i>	v
Objectives of the Workshop	
<i>A. Irvin</i>	1
Current country status reports	
East Coast fever (ECF) immunisation in Kenya	
<i>D. Kariuki</i>	5
ECF immunisation in Malawi	
<i>C. Chizonda</i>	11
ECF immunisation in Rwanda	
<i>S. Mbarubuyeke</i>	13
ECF immunisation in Tanzania	
<i>J. Melewas, M. Majaliwa and L. Lynen</i>	16
ECF immunisation in Uganda	
<i>R. Nsubuga-Mutaka</i>	26
ECF immunisation in Zambia	
<i>M. Mangani</i>	30
Immunisation against theileriosis in Zimbabwe	
<i>U. Ushewokunze-Obatolu</i>	34
Tick-borne disease (TBD) immunisation in Swaziland	
<i>R. Thwala</i>	37
TBD immunisation in South Africa	
<i>D. De Waal</i>	40
Vaccine production	
Production of <i>Theileria parva</i> stabilates for immunisation against theileriosis	
<i>F. Musisi</i>	45
The preparation of a composite stabilate for immunisation against East Coast fever	
<i>S. Morzaria, P. Spooner, R. Bishop and S. Mwaura</i>	56
Preparation of stabilates for immunisation against East Coast fever at NVRC, Muguga, Kenya	
<i>R. Payne</i>	62
Application of molecular tools in support of deployment of <i>Theileria parva</i> live vaccines	
<i>R. Bishop, V. Nene, P. Spooner, S. Mbogo, D. Kariuki, R. Payne and S. Morzaria</i>	66
Standards for live tick-borne disease vaccines	
<i>T. Dolan</i>	70

Summary of discussion: Vaccine production	
C.G.D. Brown, S. Mbogo and M. Moran	73

Vaccine delivery

The infection and treatment method of immunisation: Practice and problems	
S. Mbogo	77
Vaccine delivery in Uganda	
R. Nsubuga-Mutaka	80
Vaccine delivery in Tanzania	
M. Majaliwa, J. Melewas and G. Lynen	85
Immunisation against theileriosis in Zimbabwe using the Bolvac vaccine without oxytetracycline	
J. Mutugi	89
Practical aspects of regional East Coast fever vaccine delivery	
J. de Castro and M. Leneman	91
Commercialisation of vaccine delivery	
N. McHardy	94
Summary of discussion: Vaccine delivery	
N. McHardy, L. Lynen and T. Dolan	101

Socio-economics and impact assessment

ECF immunisation in the smallholder sector: Points to consider in funding and sustainability	
B. van Munster and L. Lynen	105
Estimating demand for theileriosis vaccines	
A. Mukhebi and T. Williams	110
Assessing the impact of ECF vaccination: Implications of structural adjustment	
A. McLeod	114
Summary of discussion: Socio-economics and impact assessment	
A. Mukhebi, J. Mutugi and E. Peeler	119

Alternative vaccines

Subunit vaccines for the control of <i>Theileria parva</i>	
A. Musoke, S. Morzaria and V. Nene	125
Multi-component subunit vaccines against <i>Theileria parva</i>	
D. McKeever	131

Training requirements for vaccine delivery

ECF immunisation: Training requirements for veterinarians and field staff	
R. Rumberia	135
Extension programme development for the delivery of East Coast fever (ECF) vaccines	
M. Moran	139

Recommendations

Recommendations	143
Summary of recommendations from Regional Tick and Tick-borne Disease Control Workshops held between 1976 and 1997	
<i>T. Dolan</i>	147
List of participants	

Preface

Theileria parva, a tick-transmitted protozoan parasite, causes a severe disease of cattle, variously referred to as East Coast fever (ECF), January Disease or Corridor Disease. The disease continues to be a major constraint to livestock production in much of eastern, central and southern Africa. The control of the disease through intensive acaricide application has been successful but is becoming increasingly unacceptable for several reasons including pollution of the environment, contamination of livestock food products and high cost. Over the past 20 years, a research programme to control ECF was developed by the Food and Agriculture Organization of the United Nations (FAO). The principal aim of the programme, funded by a number of donor agencies including the United Nations Development Programme, the Governments of Denmark, United Kingdom, The Netherlands and Belgium, was to develop a vaccine against ECF. This multi-donor programme resulted in the development of a first generation live-vaccine, referred to as the infection and treatment method of immunisation. Further research, including numerous field trials, has resulted in significant modification of the method since its first description. This evolution of the infection and treatment method, together with a better understanding of the epidemiology of the disease and the existence of parasite diversity, has culminated in the use of a number of vaccine stabilates. These vaccine stabilates are based on different *T. parva* stocks and include the 'Muguga cocktail', Marikebuni, Boleni and Katete stocks.

A series of regular formal meetings has been held to discuss progress in the control of *T. parva* and other tick-borne pathogens among the representatives of the national veterinary departments, researchers from various international and national research institutions and the donors. The first such meeting in this series was held in 1984 at the International Laboratory for Research on Animal Diseases (ILRAD, now the International Livestock Research Institute (ILRI)). Five more meetings have since been held in Malawi, Uganda and Kenya. The meeting recorded in this proceedings was held in the form of a workshop at ILRI in March 1997 and was jointly organised by the Organization of African Unity/Inter-African Bureau of Animal Resources (OAU/IBAR), FAO and ILRI. The workshop participants discussed the deployment of live ECF vaccines and focused on the problems associated with their delivery and the solutions to these problems. This proceedings provides a record of all the country presentations, invited papers, group discussions and recommendations of the workshop.

Representatives from all the countries within eastern, central and southern Africa, where tick and tick-borne diseases (T & TBDs) constitute a major economic constraint to livestock development attended the meeting. In addition, a number of scientists from the Kenyan National Veterinary Research Centre, the International Centre of Insect Physiology and Entomology (ICIPE) and ILRI participated in the workshop. The FAO and donor representatives from The Netherlands, Denmark and the United Kingdom were also represented. The workshop participants hope that the recommendations made at this

workshop will be implemented and will help stimulate positive development and progress in the control of T & TBDs.

I would like to thank all the participants from the countries in the region and representatives from international and national research institutions for their input, which resulted in the success of the workshop. The donors (Belgium, The Netherlands, Denmark and the United Kingdom), the FAO and OAU/IBAR, are thanked for their continued support and commitment to improved control of T & TBDs in the region, and the members of the organising committee for their assistance in planning and formulating the programme. Special thanks go to the Department for International Development of the United Kingdom (DFID) and OAU/IBAR for providing financial support towards the compilation of the summary of the recommendations (annexed) from all the previous meetings in this series and publication of the current proceedings, respectively. Dr Rob Eley's assistance in providing logistical support in the organisation of the workshop is also gratefully acknowledged. I also thank Joel Mwaura and Susan McMillan for their help in the design of the cover, and David Elsworth and the staff of the Graphics section in Nairobi for their input in preparing the illustrations of this proceedings.

Subhash Morzaria

International Livestock Research Institute
Nairobi
Kenya

Objectives of the Workshop

A. Irvin

Background

At a meeting of the Programme Steering Committee (PSC) of the Food and Agriculture Organization of the United Nations (FAO)/Multidonor Regional Programme on control of ticks and tick-borne diseases (TBDs) in Lilongwe in June 1996, it was agreed that this Workshop should be held to identify research needs to improve the present live *Theileria parva* vaccines and their delivery, and to consider new developments which could lead to improved vaccines.

Purpose of the workshop

1. To consider the present method of immunisation against East Coast fever (ECF) and other forms of *T. parva* infection using the infection and treatment method or infection without treatment, and to identify needs for further research to improve vaccine quality and suitability.

This will include an evaluation of production, storage and delivery techniques; vaccine production standards including quality control, safety and assurance issues; strain and stock composition of vaccines (how to identify and select strains); and specific country requirements. In addition, specific research needs will be identified and a programme for future research developed.

2. To consider present and future demands for *T. parva* vaccine and how these can be met.

This will include an assessment of present demands by country; consideration of how the demands can be met and requirements for increasing production; and projected demand for the future.

3. To review recent progress in developing alternative vaccines for the control of theileriosis caused by *T. parva*.

This will include a review of present research to develop subunit vaccines and future research needs; consideration of ways in which research programmes on present and future vaccines can be more closely integrated; prospects for alternative vaccines.

4. To consider related research needs.

This will include consideration of the need for improved diagnostics and characterisation methods; improved methods of identifying demand and target groups; improved methods of vaccine delivery; impact assessment. In this context, the workshop

will also review progress in the implementation of the recommendations of the Epidemiology Workshop held in Harare in March 1996.

5. To identify training and information needs.

This will include consideration of training needs in respect of vaccine production, handling, delivery and monitoring; improved communications and information exchange.

Outputs

1. The main output will be jointly publishing the proceedings, which will document:
 - the current status of each country with respect to ECF immunisation,
 - key areas of research to improve the quality and suitability of the vaccine, including an outline research programme
 - key areas of research for development of improved vaccines, and future prospects that such vaccines offer
 - needs for research in other areas which will help improve application and uptake of vaccine
 - training and information needs.
2. An additional output will be the opportunity for participants to exchange information and review current operations at the International Livestock Research Institute (ILRI) and the Kenya Agricultural Research Institute (KARI).

Current country status reports

East Coast fever (ECF) immunisation in Kenya

D. Kariuki

National Veterinary Research Centre (NVRC), Muguga,
P.O. Box 32, Kikuyu, Kenya

Background

Kenya has a human population of over 25 million people and covers an area of 582,670 km². Twenty per cent of the total land area has high to medium agricultural potential and is suitable for crops, intensive forestry and livestock raising. These zones support 80% of the total human population. Kenya also has an estimated 13.5 million cattle of which 3.2 million head are exotic dairy and beef cattle in large- and small-scale holdings in the high and medium potential agricultural zones. The remaining 10.3 million head are indigenous zebu cattle found in the arid and semi-arid areas, which form approximately 80% of the country. The livestock sector contributes about 10% of the country's gross domestic product, accounts for over 30% of the farm-gate value of agriculture commodities and employs over 50% of the agricultural labour force.

Smallholder production dominates Kenya's agricultural sector. There are about 3 million smallholder farms of which 80% are less than 2 ha with women providing the bulk of labour and heading a third of the households. Despite their small sizes, smallholder farms account for over 75% of total agricultural production and over 50% of marketed production. In the livestock sector, smallholders likewise account for the production of over 80% of all milk, and 70% of beef and other meat. For the small-scale farmers in Kenya, the dairy industry is therefore, a very important income-generating enterprise.

This industry is, however, beset with numerous constraints. These include inadequate water and animal feeds (fodder and pasture), high cost of concentrates, diseases requiring control and treatment, poor marketing information, diminishing land resources for livestock keeping due to increasing population pressure, poor animal husbandry practices, inadequate credit facilities for dairy farmers and poor rural infrastructure. These constraints coupled with the rapid increase in population testify that the demand for major livestock products will outstrip the current rate of production (Table 1).

Table 1. *Output and demand for beef and milk in Kenya.*

Product	Output in 1992	Demand in 2000
Beef ('000 t)	252.0	336.0
Milk (billion kg)	2.4	3.2

East Coast fever (ECF), caused by *Theileria parva* and transmitted by the tick, *Rhipicephalus appendiculatus*, poses one of the major challenges to the development of the cattle industry. Of the estimated 13 million cattle, 76% are at risk from ECF since the distribution of the tick vector correlates closely with the highest concentration of cattle, both indigenous and exotic. The disease is associated with a 10% mortality in zebu calves in ECF endemic areas and can cause up to 100% mortality in susceptible exotic and indigenous breeds. In addition, high productivity losses in animals recovering after treatment and the presence of the disease inhibits the introduction of high producing exotic and crossbred cattle in areas infested by the vector. In 1993, the Veterinary Department reported 24,000 ECF cases representing only an estimated 10% of the actual cases occurring in the field annually.

Five species of *Theileria* have so far been described in Kenya (Table 2). *T. parva* cattle-derived and maintained between cattle, manifests itself in the form of classical ECF while *T. parva* buffalo-derived when transmitted to cattle causes Corridor Disease. *Theileria mutans*, *T. velifera* and *T. taurotragi* infections usually cause mild transient fever and anaemia and are not often reported in the field. *Theileria buffeli* was recently isolated in Kenya and also causes mild transient fever in cattle.

Table 2. Species of *Theileria* recognised in Kenyan cattle.

<i>Theileria</i> species	Disease	Vector
<i>T. parva</i> (cattle-derived)	East Coast fever	<i>Rhipicephalus appendiculatus</i>
<i>T. parva</i> (buffalo-derived)	Corridor Disease	<i>R. appendiculatus</i>
<i>T. taurotragi</i>	Usually benign	<i>R. appendiculatus</i> and <i>R. pulchellus</i>
<i>T. mutans</i>	Usually benign	<i>Amblyomma</i> spp
<i>T. velifera</i>	Benign	<i>Amblyomma</i> spp
<i>T. buffeli</i>	Usually benign	?

Since 1904, when the disease was first recognised in Kenya, the strategy for tick and tick-borne disease (T & TBD) control in Kenya has been predominately based on controlling *R. appendiculatus* through intensive application of acaricides. The acaricides are applied once or twice a week to prevent transmission of *T. parva* and to allow introduction of improved exotic breeds of cattle and upgrading of the indigenous cattle population in ECF affected areas. However, in the past decade or so, it has become apparent that intensive tick control using acaricide is becoming prohibitively expensive. In addition, strict acaricide application that prevents any contact of cattle with ticks does not allow calves to develop immunity to TBDs other than ECF at an early age. In addition, the method does not allow cattle to develop immunity to ticks. Thus, strict acaricide application creates endemic instability as animals remain susceptible to tick-borne diseases. If for any reason the dipping programme is interrupted, there will be a potential danger of a TBD upsurge of epidemic proportion.

With the introduction of the infection and treatment method of immunising cattle against ECF, however, it should be possible to relax the frequency of acaricide application.

Progress on East Coast fever immunisation

Currently, the only available method of immunisation against ECF requires injecting an animal with a potentially lethal parasite preparation together with a therapeutic dose of a long-acting oxytetracycline. A small proportion of the immunised animals may develop the disease and must be treated. These animals are commonly referred to as reactors and are identified through follow-up monitoring between days 10 and 14 after immunisation.

The major costs associated with this method of immunisation include those of the oxytetracycline, monitoring exercise, antitheilerial drugs to treat reactors and the possible loss of milk due to milk withdrawal after administering drugs to lactating cows. The cost of treating a reactor animal completely negates the cost benefit of immunising the animal as the farmer has to cover the full cost of treatment. Despite antitheilerial treatment, the animal could also die leading to litigation and subsequent compensation.

Over the past few years a lot of human and financial resources have been invested in developing an ECF vaccine. The main objective of the research and field trials in Kenya has been to produce a vaccine that is affordable, safe, effective, easily manageable and acceptable to the user. The other important aim is to produce an acceptable integrated T & TBD control package for the farmer.

Cost reduction

- Research has confirmed that oxytetracycline preparations much cheaper than those used previously, are equally effective. This is important in animal health since oxytetracyclines are used to treat other livestock diseases.
- Follow-up monitoring has been reduced from daily to once every three days.
- Calves can be successfully immunised from one month of age. Calves are easier to handle and require less of the drug. They also run no risk of milk withdrawal or abortions if they react.
- A *T. parva* isolate of low pathogenicity has been identified which provides a broad spectrum of protection. Due to its low pathogenicity, if it were used for immunisation the administration of an oxytetracycline would be unnecessary and the risk of reactors low making follow-up monitoring redundant. The isolate is also known to produce low schizont parasitosis with no clinical reaction.

Making the procedure more manageable

Stabilates are stored in 0.5 ml straws, which are much easier to handle than the 2.8 ml vials previously used. It has also been shown that the stabilate is infective after 24 hours and

protective after 4 hours when kept on ice. The field staff can reconstitute the stabilate and use it for the next four hours instead of just one hour. This has eliminated the need to carry ice buckets and liquid nitrogen containers to the field, both of which are not readily available.

Making the procedure safer

The stabilate undergoes quality control in the laboratory to avoid transmission of other diseases. Quality control includes titration studies to determine a safe but protective level of dose. During the field trials, it has been shown that immunisation with the current parasite does not precipitate clinical cases of other TBDs, a fear expressed because ECF is immuno-suppressive. Since 1987, when the field trials started, 5729 cattle have been immunised in various parts of the country (Coast, Rift Valley, Central and Western provinces) and none have contracted extraneous diseases associated with the stabilate prepared at the National Veterinary Research Centre (NVRC), Muguga.

Staff training

An ECF immunisation-training manual has been prepared and an elaborate training is given to the immunising personnel together with information on how to handle the risks involved. As a precaution, the long-acting oxytetracycline is administered before the stabilate in case cattle escape during the immunisation.

Stabilate production

Currently, a large volume of the immunising stabilate (*T. parva* Marikebuni), enough to immunise 120,000 cattle, has been produced, titrated and tested. This stock has been supplied to a group of recently-trained veterinarians for immunisation in smallholder dairy farms in Coast Province.

Country perspectives

The system has proved effective at the Coast with farmers willing to pay for immunisation. Demand for immunisation at the Coast is quite high. Farmers have asked for the immunisation through their veterinarians who have been trained by staff at Muguga and have immunised around 600 animals with minimum logistical support from the laboratory. The farmers have accepted the technology more readily than the professionals (field veterinarians). Smallholder dairy farmers in Kiambu District (Central Province) are also quite willing to have their animals immunised.

During 1994 and 1995, a number of field staff were trained and approximately 2250 cattle were immunised in various ecological zones for *T. parva* (Marikebuni). Sixty-five calves aged between one and seven months were also successfully immunised at the Coast.

To prove that the procedure is cost-effective, a model has been developed to compare the financial implications of immunisation of calves, immunisation of whole herds, treatment of clinical cases without immunisation and a no immunisation option. These factors are considered before immunisation is undertaken.

Experience during the past few years has shown that, for the ECF immunisation programme to succeed, it should have an economic consideration. Two criteria seem to be necessary for immunisation to succeed. These are ECF should be a major production constraint to the farmer and that there are veterinarians in private practice or government employment who are willing to be trained to deliver the ECF immunisation.

For ECF immunisation by the infection and treatment method to be a sustainable technology, the beneficiaries (the small dairy cattle owner and others) have to be willing to take the risk of possible reactors as the present vaccine stabilate is potentially pathogenic. The veterinarians need to have the ability to immunise with confidence.

Country needs and policy

The current government policy promotes a shift from subsidised services to increased cost-sharing and, eventually, to full-cost recovery and privatisation of some aspects of veterinary services including management of dips. Although ECF is a notifiable disease, immunisation against the disease is not amenable to mass campaigns such as those conducted for rinderpest or Foot-and-Mouth diseases. The disease affects individual animals and vaccination will depend on the willingness of the farmer to pay for it. With the escalating costs of acaricides and the environmental issues related to their use, immunisation offers an alternative method of controlling the disease. Although ECF is the major constraint to the dairy industry, the policy views immunisation against the disease as a private good putting minimal pressure on the farmer to immunise his or her cattle. The policy however, gives the Director of Veterinary Services the overall responsibility of guiding where immunisation is to be carried out. The Director is also responsible for monitoring the immunisation services both by private veterinary practitioners or those in the civil service in their private capacity and ensuring that the delivery process is carried out in accordance with regulations governing disease control in the country.

The stabilate currently being used in field trials was produced at NVRC, Muguga, and it is expected that this laboratory will continue with that role for the country.

With the above in mind, immunisation against ECF in Kenya could be guided by the following principles:

1. To immunise cattle through private approaches in areas where field trials have shown that the current stabilate is efficacious and farmers are willing to have their cattle immunised.
2. To continue producing the stabilate at the laboratory in Muguga and conduct research to improve the vaccine. Such research should include the assessment of other *T. parva* stabilates that do not require the use of tetracycline. The capacity of the national laboratory to produce stabilate and conduct research and development must therefore,

continue to be strengthened to ensure appropriate, effective and efficient technical support to the immunisation programme.

3. To continue to train veterinarians in the private sector and in the government service and strengthen the infrastructure to ensure that immunisation is carried out with confidence. This includes production and dissemination of information about ECF immunisation and control of other TBDs to veterinary professionals, the extension services and farmers. The field veterinary staff should likewise have adequate facilities to make accurate parasitological diagnoses.
4. To continue research to understand the epidemiological implications of ECF immunisation so that farmers can be given information that allows them to use less acaricide to control ticks and other TBDs that may be present.

Proposals for Kenya

- The full benefits of ECF immunisation will only be realised if it is accompanied by reduced acaricide application to control ticks through strategic tick control methods. For immunisation to succeed the area selected should therefore have very good tick control with functional dips using effective acaricides at the correct strength. In some parts of the country, since *R. appendiculatus* co-exists with the vectors of other TBDs, there is a need to establish the incidence and severity of other TBDs, their distribution and seasonality and also the tick species involved. The Project should therefore support tick control by providing seed acaricide and funds to generate the above information to help advise on strategic tick control,
- The Project should strengthen and support research on improving the vaccine at NVRC, Muguga,
- The Project should support vaccine delivery in selected parts of the country.

ECF immunisation in Malawi

C. Chizonda

Animal Health and Industry, P.O. Box 2096,
Lilongwe, Malawi

Introduction

East Coast fever (ECF) is endemic in the central and northern regions of Malawi, while the southern region is free of the disease although the vector is present. Higher mortality is observed in calves than in adult animals.

In the past, Malawi controlled ECF by dipping animals every week, at times twice a week, in an attempt to control the vector. Due to economic constraints however, the Veterinary Department was forced to review its dipping policy in 1993 and a tick ecology survey was undertaken. Using the results of the survey, the department recommended dipping animals fortnightly between November and April. However, in areas where nymphal transmission occurs after April, due to a prolonged wet season in some parts of northern Malawi, it has been recommended that dipping should continue beyond April.

In recent years, irrespective of this change in the policy, animals have not been dipped as recommended because of lack of funds to buy the required acaricides. Instead, some individual livestock farmers have resorted to applying their own tick control methods using pour-on preparations or dip wash. Farmers with valuable animals, however, have combined vaccination with tick control using pour-on preparations to control ECF.

Currently, there is an urgent need to study the impact of the past few years of intermittent dipping on the health and productivity of the livestock population and particularly those indigenous cattle which predominate in rural areas.

Following an Organization of African Unity/Scientific, Technical and Research Commission (OAU/STRC) resolution in Algiers (October 1976) to establish regional centres for the control of tick-borne diseases (TBDs), a TBD control centre was set up at the Central Veterinary Laboratory in Lilongwe, Malawi, for countries in eastern, central and southern Africa.

Subsequently, Malawi requested assistance from various donors to support the initiative. A series of projects with an overall objective of creating a self-supporting centre for the control of ticks and TBDs has since been implemented by the Food and Agriculture Organization of the United Nations (FAO) between 1979 and 1993. The initiative culminated in the establishment of the vaccine production centre (VPC). The VPC was formally opened in April 1994 after vaccine production laboratories were built.

Current status of the VPC

Shortly after its inauguration the VPC encountered several problems, including the loss of 4 of its 5 external technical assistance staff and nearly half of its national technical support staff later in the year. Vaccine production was then indefinitely suspended due to concerns that products from the centre might not be of satisfactory standard, despite indications that farmers and veterinarians in the field were happy with the vaccine. To bring activities of the VPC to a pre-closure level will require firm commitment from personnel and continued funding of the various operations. Revitalising the VPC seems to be the desire of the recipient countries which hope that this can now be initiated for the greater benefit of the region.

ECF immunisation in Rwanda

S. Mbarubuyeke

National Veterinary Laboratory, P.O. Box 804, Kigali, Rwanda

Introduction

Tick-borne diseases (TBDs), especially East Coast fever (ECF), are a major constraint to the development of improved livestock systems in parts of Rwanda. They are estimated to account for 30% of young and 9% of adult mortality in improved dairy breeds. Before the Rwandan civil war of 1994, ECF posed a major obstacle to the implementation of livestock projects using crossbred or pure-bred cattle other than local Ankole. As a result of the civil war, the Rwandan veterinary service has lost many of its facilities and personnel. However, various efforts are well under way on the part of the government and private farmers to initiate new and rehabilitate existing livestock improvement projects. These projects are based on the premise that ECF is a major obstacle to the development of the livestock industry in Rwanda.

This report describes the nature of the cattle population of Rwanda and highlights the different measures currently applied to control ECF. The author also presents the salient features of the national project for integrated tick and tick-borne disease control (ITTBD project) for Rwanda, the result of an agreement between the Government of Rwanda and the Food and Agricultural Organization of the United Nations (FAO) which is being considered for funding.

Cattle population of Rwanda

The number of cattle in Rwanda, including improved milking cattle, was drastically reduced during the 1994 civil war. From 1994 onwards, milking breeds (Friesian, Brown Swiss) have been imported and large Ankole herds have entered Rwanda from neighbouring countries.

The total cattle population is estimated to number approximately 600,000 head of which 5% are presumed to be improved dairy breeds. Thus, an estimated 30,000 animals could be potential targets for ECF immunisation.

Disease diagnosis and control

The disease diagnosis and control potential of the Rwandan veterinary service has also fallen victim of the civil war. The National Veterinary Laboratory of Rubilizi has been rehabilitated and has now begun to carry out disease diagnosis, which for ECF involves

Giemsa staining and serology. Apart from ECF, other important diseases include contagious bovine pleuro-pneumonia (CBPP) and helminthiasis. To tackle these, the veterinary services are being improved. However, some important sections of the laboratory, including the bacteriology department are not yet operational.

Acaricides are still being used to prevent ECF and other TBDs. When disease occurs, ECF is treated using buparvaquone. However, this has proved very expensive with a 40 ml bottle costing 18,000 Rwandan francs (US\$ 60).

Widespread vaccination is implemented for the control of CBPP and deworming programmes to control helminthiasis are undertaken on a massive scale twice a year. Although, there is a net improvement in livestock activities, much remains to be done in terms of providing veterinary services in remote areas that often depend on help from non-governmental organisations (NGOs).

The ITTBD Project for Rwanda

Following the Lilongwe meeting of June 1995, Rwanda has made a formal request for assistance through FAO representation to mobilise existing funds (US\$ 12,754) allocated to the Rwanda national project, which failed to start in 1994. Rwanda is reliant on the release of these project funds for the effective control of ECF and to implement a vaccine programme. With the collaboration of the Ugandan ITTBD project, the Rwanda project description has been revised and adapted to reflect the present situation. The Ministry of Agriculture and Livestock has submitted a funding request to FAO.

The main activities to be implemented by the project are:

- To test the efficiency of the Muguga cocktail on cattle reared in Rwandan conditions. Selected local *T. parva* strains, which were to be used to immunise the animals, were unfortunately lost during the war due to lack of liquid nitrogen. Male calves will be imported from Uganda and raised in Rwanda to test the Muguga cocktail. Most, if not all, the pure- and crossbred milking cattle have been imported from Uganda, and there would not be enough calves available in Rwanda for use as experimental animals.
- To train veterinarians and animal health technicians in the correct methods for ECF immunisation so that the necessary personnel is available to do the immunisation once the project starts in Rwanda.

The project is scheduled to last 9 months with a possible extension phase, if the outcome of the immunisation trials is satisfactory and the Muguga cocktail causes no harm to cattle in Rwanda.

Conclusion

After the war of 1994, some encouraging results have been registered in the effort to rehabilitate the livestock system though much remains to be done. Any successful use of improved dairy cattle in Rwanda presupposes an effective ECF and other TBDs control

programme. This will involve acaricide use coupled with immunisation. A request for rapid funding of a national project for tick and tick-borne disease control, which was originally due to start in 1994, has been submitted to FAO.

ECF immunisation in Tanzania

J. Melewas,¹ M. Majaliwa¹ and L. Lynen²

1 Livestock Development, P.O. Box 9152, Dar es Salaam, Tanzania

2 Veterinary Investigation Centre, P.O. Box 1068, Arusha, Tanzania

Background

Tanzania covers an area of 945,027 km² with an estimated 26 million people. According to the National Sample Census of Livestock by the Ministry of Agriculture in 1994/95, Tanzania has more than 15.6 million cattle, 98% of which are found in the traditional sector. Cattle dominate the livestock industry of Tanzania. Despite the large population and vast rangeland resources, their contribution to both agriculture and national gross domestic product is relatively low. The major constraints to the development of the livestock industry in Tanzania include poor nutrition and husbandry, genetic limitations, an unco-ordinated marketing system and disease.

Vector-borne diseases, grouped into tick and tick-borne diseases (TBDs), constitute a major obstacle to the industry. East Coast fever (ECF) is the major cause of cattle deaths and losses in livestock production, followed by tsetse and trypanosomosis. Animal disease control in Tanzania has been prioritised according to these observations. The main form of control of ECF has been the use of acaricides, either by dipping or spraying. The Germans built the first dip in 1905 at Mwapwa and a country-wide network of facilities has since been constructed totalling 2100 plunge dips. However, most proved inefficient and suffered a number of other problems including poor dip management, high acaricide costs, unreliable dip wash testing, and the emergence of acaricide resistant tick strains; these dips are not currently operational. While hand spraying or pour-on acaricides are commonly used in the smallholder dairy sector, their application is irregular and not entirely efficient.

A joint Food and Agriculture Organization of the United Nations (FAO)-United Nations Development Programme (UNDP) programme for East Africa for the control of ticks and TBDs was initiated in 1967 (UNDP Project RAF/67/007). The Tanzanian component was project URT/72/009. In its final phase, this project conducted an important trial on the use of the Muguga cocktail in experimental cattle held at Pugu holding ground outside Dar es Salaam. One of the recommendations in the final report of this project was the 'routine use of this method of immunisation against ECF on a large-scale to protect valuable animals at risk, combined with additional immunisations against other TBDs where deemed necessary' (Uilenberg 1977).

With the collapse of the East African Community in 1977, United Nations funding ceased. FAO, which had been the executing agency for the UNDP-financed projects in Kenya, Uganda and the United Republic of Tanzania, obtained support from the Danish

International Development Agency (DANIDA) in 1980 for a series of projects in Malawi, Zambia, Zimbabwe and Kenya.

Tanzania was left out of the project until 1990 when DANIDA reintroduced the programme (document GCP/URT/098/DEN). The Tanzanian government in November 1990 approved a revision of this project document. The programme was extended up to October 1993, and a follow-up project (document GCP/RAF/299/NET) covered activities to the end of February 1997.

Objectives of the project

The overall objective of the Regional Programme for East Africa was: 'The development of a cost-effective strategy for the control of ticks and tick-borne diseases in the region.'

Phase I of the programme saw the development phase of the immunisation method, while Phase II involved the field application and expansion to other countries in the region. Together the two phases led to the establishment of a central tick-borne disease vaccine production unit (VPU). The overall objective of Phase III was to 'ensure the availability of appropriate methods, including vaccination throughout the region' by manufacturing sufficient quality vaccine and testing the delivery systems at an economically sustainable cost.

The immediate objectives of the Tanzanian national component were to evolve an effective, sustainable and environmentally friendly integrated tick and tick-borne disease control programme by:

- introducing a sustainable delivery system for immunisation against TBD in Tanzania, eventually to be used with reduced acaricide application
- establishing a system for monitoring the effectiveness and socio-economic impact of the immunisation programme
- training national staff with the aim of handing over the above activities.

The long-term objective of this project was to improve the general standard of living through the improved health and productivity of cattle in the country.

Immunisation activities

Tanzania mainland

From 1990 to February 1997, animals were immunised using different batches of the trivalent vaccine (Muguga cocktail) supplied by the VPU. A total of 14,628 dairy animals and their crosses (4.5% of the improved high-grade cattle) were immunised and monitored by project staff for 30 days after immunisation. Of a total of 576 animals, 3.9% were reactors to immunisation and 0.27% of these died during the monitoring period, while 4 animals (0.03%) died of anaphylactic shock immediately after immunisation (Table 1).

Table 1. Summary of number of animals immunised and results of monitoring by region.

Region	No. immunised	ECF reactor		Anaphylaxis		ECF deaths	
		No.	%	No.	%	No.	%
Iringa	6993	209	2.9	3	0.04	26	0.37
Mbeya	364	0	0	0	0	0	0
Morogoro	348	23	6.6	0	0	3	0.86
Dar and Coast	1379	18	1.3	6	0.45	4	0.29
Tanga	295	7	2.4	1	0.34	3	1.02
Kilimanjaro	529	129*	24.4	0	0	5	0.95
Arusha	1186	89	7.5	50	4.22	4	0.34
Mwanza	2737	98	3.6	1	0.04	8	0.29
Kagera	797	3	0.4	0	0	1	0.13
Total	14628	576	3.9	61	0.42	54	0.37

*Includes 44 calves which reacted during the October 1996 immunisation; calves were dewormed one week post-immunisation and developed diarrhoea during the monitoring period.

The number of reactors occurring appears to be seasonal only in Morogoro and Iringa regions. This could be linked to concurrent ECF field challenge and the tick-control method of the livestock owner, since tick control is neglected during the months July to September when adult ticks are virtually absent, leaving the cattle exposed to nymphal challenge.

In all regions, the most obvious reason for a high or low number of reactors is directly linked to the experience of the delivery veterinarian. Areas with long-standing activities report low numbers of immunisation reactors, while individuals undertaking the delivery for the first time tend to begin with 8–15% reactors and decrease to 3% or less after a 6–8 month period.

An unexpectedly high number of anaphylactic shock cases were recorded in Karatu and Mbulu Districts (Arusha region) between September and November 1996. The animals were treated with adrenaline (1/1000 at 0.1 ml/kg IV) and recovered. It is presumed that the tick antigen in the stabilate causes anaphylactic reactions. This high number could thus be linked to severe exposure to *Rhipicephalus appendiculatus* during the previous rainy season when up to 30% losses due to TBDs, particularly ECF, were recorded in the dairy population of these districts.

Tanga coastal region

Tanga region supports over 600,000 cattle, most of them indigenous Tanzanian zebu, under traditional management. Approximately 12,000 grade dairy cattle are present, under zero- or semi-zero grazing management. ECF is the most important cause of adult and calf mortality in highgrade dairy cattle in the region. Existing tick control practices are expensive and not 100% effective. The Tanga Smallholder Dairy Development Project was willing to

adopt ECF immunisation using the trivalent vaccine provided protection against the prevalent field strains could be demonstrated.

To formulate an integrated ECF control strategy for Tanga region, a series of activities were undertaken:

- assessment of the efficacy of the trivalent vaccine
- sero-survey to determine the target groups for immunisation and the potential risk of other TBDs
- monitoring of previously immunised animals
- assessment of the interest of farmers in adopting new control strategies.

Immunisation trials

Three trials were conducted between January 1995 and November 1996 to determine the efficacy of the trivalent vaccine. Results of the first trial initiated by the Tanga Dairy Project were inconclusive and no serology result reports were available pre- and post-immunisation. In the second trial (October 1995–April 1996) only a few deaths due to ECF occurred in each group: 3 out of 20 immunised animals and 2 out of 12 control animals. Six clinical cases of heartwater (HW), three of which died, complicated the clinical presentations in a number of animals. Serology pre- and post-exposure showed 100% sero-conversion to *Cowdria ruminantium*. The results of this trial were inconclusive. A third trial in July 1996, synchronised the period of field exposure with the season of high ECF challenge (August–September). Acaricide application (tick grease) at the predilection sites of *Amblyomma* spp was used in an attempt to avoid complications with HW during exposure. The average number of *R. appendiculatus* during field exposure ranged between 150 and 280 ticks per animal (Table 2).

Table 2. ECF immunisation and field exposure trial 3: July 1996–November 1996.

	Total no.	NR/MR	SR/FR	Overall mortality	Cause of death			
					ECF	ECF/HW	HW	Other
Immunised	15	8	7	8	3	1	1	3
Controls	14	7	7	13	7	0	1	5

Significance Fisher Exact $p=0.57$ $p=0.02$ $p=0.10$.

NR = non-reactor; SR = severe reactor; MR = moderate reactor; FR = fatal reactor.

A significant difference [$p=0.02$] was found in the overall survival rate between the immunised and the control animals. However, the number of deaths due to ECF, although higher in the control group, is not significantly different [$p=0.10$] from the number recorded in the immunised group. Given these results, it appears that in Tanga, the trivalent vaccine elicits partial protection of between 25% and 75%.

Isolation of local *T. parva* strain

An attempt to isolate a local strain during the first trial in Tanga failed (Musisi 1995). During the second trial, another attempt was made by applying clean laboratory-reared *R. appendiculatus* nymphs to three clinically sick control calves. Tick engorgement, harvesting and subsequent moulting was successful and the resultant adult ticks were sent to the International Livestock Research Institute (ILRI) for stabilate production and further characterisation. Although the parasitaemia in the calves at the time of nymphal feeding was relatively low (4–6%), a high tick infection rate was observed. Stabilate production (stabilate 4134) was successful and characterisation is under way. In view of the unsatisfactory results of the Tanga immunisation trials using the trivalent vaccine, this local isolate may have potential for use in Tanga region and merits further investigation.

Cost recovery for delivery activities

Cost-recovery was initiated in 1992 after encouraging results were achieved with immunisation activities in southern highlands during Phase II (Project GCP/URT/098/DEN). The vaccine package was handed over to delivery veterinarians at a price of Tanzanian shilling (Tsh) 1000 (US\$ 3.1) per dose, which was later raised to Tsh 1500 (US\$ 4.1) in 1993. At the National Steering Committee Meeting, held in Morogoro in February 1996, a proposal to once again review the price of the vaccine package and move gradually forward to full cost-recovery was accepted and permission was given to open additional ECF accounts in the regions to facilitate collection of revenue. The package price has gradually increased and now stands at Tsh 4000 (US\$ 7) per adult animal and Tsh 3000 (US\$ 5) per calf. A final price increase is expected in March 1997, to reach full cost-recovery of the vaccine package. Veterinarians have been allowed to fix their own farm-delivery price, which ranges between Tsh 5500 and 10,000 (US\$ 9–17). No major problems have been experienced in introducing this level of pricing in the areas where activities have begun recently. Cost-recovery was more readily accepted in areas where ECF causes high losses in the dairy population. Most outstanding debts are related to Government Livestock Multiplication Units (LMU), which did not budget in anticipation of immunisation activities and have subsequently experienced difficulties in releasing payment for immunisations carried out on their farms.

ECF immunisation follow-up activities

Serology

Serology data have been compiled from serum samples collected from smallholder farming systems, commercial farms and traditional indigenous herds. Sera were tested by the indirect fluorescent antibody test (IFAT) using *T. parva* schizont antigen, and, when possible, also by the enzyme-linked immunosorbent assay (ELISA) test at ILRI. The majority

(83%) of serum samples were collected from adult animals. Routine pre- and post-immunisation serum sampling was undertaken in most regions. Pre-immunisation seroprevalence for *Theileria parva* ranged from 0% to 40%. Seroconversion 30 days after immunisation ranged between 57% and 100%. Pre-immunisation serum samples from commercial farms were tested for antibodies to the major TBDs (ECF, anaplasmosis, babesiosis) to formulate a rational tick control strategy after immunisation. Results clearly indicated that endemic stability existed for anaplasmosis on most of the farms, with seroprevalence ranging between 21% and 77%, while for babesiosis an endemically unstable situation was found with seroprevalence ranging between 0% and 36%. Detection of animals seropositive to *Babesia bovis* using the IFA test in the northern and lake zone regions was surprising as its vector, *Boophilus microplus*, has only been identified in the southern highlands and parts of Dar es Salaam and Tanga coastal area (Yeoman and Walker 1967). Confirmation of clinical disease due to *Babesia bovis* was made in Arusha (Imani Estate) and Mwanza (Mabuki farm) and further investigations are warranted to establish the new geographical range of this disease and its vector.

Adjustment of tick control

The high cost of acaricides has been the most important reason for commercial farmers to take on ECF immunisation. Therefore, a revised tick control practice had to be formulated to allow for a reduction in acaricide costs and to increase the chances of boosting immunity naturally in immunised animals. Half-body tick collections are carried out in all the regions to investigate tick loads and tick dynamics during the year. Depending on the TBD serology profile of the farm, the acaricide formulation in use and the tick dynamics (based on tick collection records), a 25–50% reduction in acaricide use was recommended as being safe, while a further reduction (threshold applications) was recommended if no problems were experienced during the period of high tick challenge (rainy season). Most farmers have reduced acaricide applications by up to 50%, while others have gone from dipping twice a week with organophosphate formulations (OPD) to once a month dipping without reporting any problems.

Epidemiology studies

Serological survey in Tanga coastal region

A serosurvey was carried out in Tanga coastal region in April 1996 with the objectives of selecting target populations for ECF immunisation, assessing levels of exposure of animals under different management systems and investigating the possibility of lack of natural challenge after immunisation in zero-grazing animals. Fifty-three farms were visited and serum samples from approximately 350 animals have been tested to assess seroprevalence rates of TBDs under different management systems. Results so far indicate a situation of endemic stability for anaplasmosis in every farming system and, in most farms, an unstable situation for *Babesia bovis* and *T. mutans*. Endemic stability for ECF was recorded in the

indigenous population and most of the smallholder farms practising free grazing, while an unstable situation was found on the majority of zero-grazing farms. All samples were tested by IFAT against *T. parva*, *T. mutans* and *Babesia bovis*, while only a proportion was tested against *Anaplasma* (Table 3).

Table 3. Breakdown of seroprevalence to *Theileria parva* (T.p.), *T. mutans* (T.m.), *Babesia bovis* (B.b.) and anaplasmosis (An) by farming system.

Positive (%)	Zero-grazed farms (no.)				Semi-zero-grazed farms (no.)				Traditional herds (no.)			
	T.p.	T.m.	B.b.	An	T.p.	T.m.	B.b.	An	T.p.	T.m.	B.b.	An
0–10	19	26	27	0	1	2	3	0	0	0	1	0
11–70	16	13	11	5	4	5	5	1	1	3	2	0
71–100	6	2	3	1	4	2	1	7	2	0	0	2
Total	41	41	41	6	9	9	9	8	3	3	3	2

Low seroprevalence for *Babesia bovis* was unexpected, as *Boophilus microplus* has been identified in this region. Tick control may have disrupted endemic stability for *Babesia bovis*, although very few clinical cases are reported. This could indicate effective tick control where *Babesia bovis* infection rates are reduced to very low level, with minimum losses being recorded.

Heartwater serology

As limited information was available to assess the presence and risk of heartwater in Tanzania, serology was attempted with assistance from the United States Agency for International Development (USAID) heartwater project in Harare, Zimbabwe. A representative sample of sera from different regions was sent to Harare, together with collections of live *Amblyomma* spp from Tanga and Arusha regions to try and isolate *Cowdria ruminantium*. All serum samples were tested in the MAP-1B ELISA which is more specific than Western blotting, although cross-reactions with *Ehrlichia canis* still occur. The results implied that endemic stability exists in most of the regions where *Amblyomma* species are present and that on most of the farms, calfhood exposure is ensured.

For most regions where *Amblyomma* spp are present, relatively low seroprevalence was recorded. However, it has been observed that heartwater seroprevalence in endemic areas may be low using the MAP -1B ELISA test (Dr S. Mahan, personal communication) and this is thought to occur because animals down-regulate their antibody response after repeated challenge with *Cowdria ruminantium*. Further investigation is required, especially following conclusions by the Tanga study that heartwater can be a serious threat to improved livestock.

Recommendations

Control of ticks and TBDs can no longer be achieved by relying on acaricide application alone. An integrated approach combining tick resistant cattle, creation of enzootic stability, immunisation, chemotherapy and strategic acaricide use is recommended.

Immunisation

Immunisation of high-grade and crossbred cattle against ECF by the infection and treatment method has been carried out successfully. The results of immunisation and follow-up activities and data analysis have resulted in the following recommendations:

Smallholder dairy farming systems

In ECF endemic areas, immunisations should be carried out to protect all susceptible animals. Adjustment of tick control is not advocated as it is already done erratically and inefficiently. *Anaplasma* vaccination is not recommended but *Babesia bovis* and heartwater immunisation may be considered in the future depending on the results of further work.

Commercial dairy and beef farms

ECF immunisation of adults and calves is recommended together with reduced acaricide use. A 50% reduction can be justified as safe and further reduction will depend on the results of TBD studies at farm-level. *Anaplasma* vaccination is not recommended. Exposure of calves to ticks and TBDs should be maximised to increase endemic stability for heartwater and babesiosis.

Traditional herds

It would be unwise to embark on ECF immunisation in traditional herds if offtake is not guaranteed. Cross-boundary movement of traditional herds also makes it almost impossible to organise the logistics for an initiative at national level. ECF immunisation of calves in the traditional sector will only be initiated if progress has been achieved in the livestock marketing sector and regional activities have begun in the traditional sector.

Tick control

The vast number of different acaricides currently on the market will make it difficult to provide national guidelines for adjusted tick control. New legislation to control acaricide importation and stricter supervision of its use is needed. Routine tests on the strength of acaricides in use should be carried out and pharmaceutical companies should be obliged to provide the recommended test kits.

Training

Delivery veterinarians and paraveterinarians should be trained on an individual basis. Institutional capacity should be built up by developing local expertise, further training of national experts and supporting diagnostic and training facilities.

Co-ordination

Those involved in tick and TBD control need contact and co-operation with relevant institutions and projects both within Tanzania and elsewhere. Co-ordination of investigative activities should be strengthened between existing projects at Sokoine University of Agriculture (SUA) and the Animal Disease Research Institute (ADRI) in Dar es Salaam as well as with international groups, such as ILRI and the USAID HW project. Extension of project activities as well as data collection should capitalise on existing extension networks, especially, those of dairy projects. Open dialogue is recommended between existing projects working on tick and TBD control and any prospective donor-funded livestock project. It may thus be necessary to establish a tick and TBD unit to co-ordinate and act as counterpart agency to all related projects. Activities on Zanzibar and Pemba should be incorporated into the mainland programme or free exchange of information and better collaboration should be guaranteed.

Delivery and cost recovery

Adequately trained personnel should perform vaccine delivery at full cost-recovery. Administrative arrangements for the management of revenue from cost-recovery should be strengthened, preferably by establishing a decentralised livestock-development fund.

Immediate research needs

More research is required in some areas to support ongoing immunisation activities:

- defining the distribution of *Babesia bovis* and its vector, *Boophilus microplus*
- conducting heartwater studies, which will require expert laboratory assistance
- if immunisation failures are recorded, a local vaccine strain may be needed; investigations should clarify the potential of the local isolate for use alone in its area of origin or for incorporation into the trivalent vaccine
- economic impact assessment of the different control methods used should be an integral part of any future project proposal, as results will be a valuable tool in promotional and extension activities
- alternative effective and acceptable vaccines against ECF and other TBDs should be developed with commercial and private sector participation in production, marketing and distribution.

References

Musisi F.L. 1995. Report of tick unit (17/7/95) on tick collections from Amboni holding ground of Tanga region. (Draft)

- Uilenberg G. 1977. Studies on Theileriidae (Sporozoa) in Tanzania. A large-scale field trial on immunisation against cattle theileriosis. *Tropenmedizin Parasitologie* 28:499–506.
- Yeoman G.H. and Walker J.B. 1967. *The Ixodid Ticks of Tanzania*. Commonwealth Institute of Entomology, London, UK.

ECF immunisation in Uganda

R. Nsubuga-Mutaka

Livestock Research Institute (LIRI), National Agriculture Research Organisation (NARO),
P.O. Box 24, Entebbe, Uganda

Introduction

Land-locked Uganda covers a total of 241,000 km² including 44,000 km² of inland water. The human population is approximately 20 million. Over the past few years, the country registered a remarkable increase in gross domestic product (GDP) from 1% in 1986 to 8% in 1996. Agriculture remains the mainstay of the economy where 88% of the population is rural. Agriculture contributes 50% of the GDP and over 90% of the country's export earnings. An integral part of the agricultural sector, livestock accounts for 8% of the GDP.

The livestock population is estimated at 5.3 million cattle, 6.2 million goats, 1 million sheep, 1.5 million pigs and 22 million poultry. Animal disease is the main constraint to livestock production in the country. Ticks and tick-borne diseases (TBDs), in particular, are major impediments to the introduction of improved livestock to farms and cause high mortality in exotic cattle. Ticks infest all the 39 districts of Uganda, the most prevalent being *Rhipicephalus* spp. It transmits East Coast fever (ECF) which constitutes approximately 67% of the clinically reported TBD cases in the country. *Boophilus* ticks transmit both anaplasmosis and babesiosis which together make up 21% of the clinically reported cases; *Amblyomma* spp are vectors for heartwater which accounts for the remaining 12% of reported TBD cases.

The control of ticks and TBDs in Uganda has depended heavily on the use of acaricides, which have inherent problems of being expensive, creating tick resistance, polluting the environment and leaving residues in food. Worse still, over 70% of the dips are in a state of disrepair contributing to the failure of acaricides to control ticks.

ECF immunisation project

Immunisation against ECF using the infection and treatment method in Uganda was first carried out in the early 1970s under the auspices of the East Africa Veterinary Research Organisation, Muguga, Kenya. A study was carried out at the Animal Health Research Centre, Entebbe, where zebu cattle were immunised using separate inoculations with three isolates; *Theileria parva* Muguga, *T. parva* Entebbe I and *T. parva* Entebbe II. In spite of a very heavy ECF challenge and the concurrent incidence of other TBDs, the ECF vaccinates were found to be significantly protected as compared to the controls.

Uganda later joined the Danish International Development Agency (DANIDA) / Food and Agriculture Organization of the United Nations (FAO) Regional Programme for Ticks

and TBDs during the second phase in 1990 along with Burundi, Tanzania, Malawi, and Zambia with the objectives of evaluating the efficacy of the Muguga cocktail vaccine. The long-term aim for the Ugandan project was to develop and evaluate cost-effective strategies for the control of ticks and TBDs in Uganda. The specific objectives were to assess the protection afforded by the Muguga cocktail against Ugandan field stocks, to isolate breakthrough stocks and, if necessary, incorporate them into the vaccine cocktail and, to identify future strategies required to achieve the long-term objectives for the control of ticks and TBDs.

The primary objective of evaluating the Muguga cocktail vaccine for use in Uganda was achieved by four immunisation trials in which protection was recorded in excess of 80% of the vaccinated cattle. Consequently, it was recommended that Uganda incorporates ECF vaccination as a major disease-control strategy, to be carried out in an integrated approach towards the control of ticks and TBDs.

Present state of the ECF immunisation project

Following the recommendation to incorporate ECF vaccination as a major disease-control strategy, the ECF immunisation programme was extended to private farms with effect from August 1994. The programme was systematically conducted beginning with the training of veterinary staff and private veterinary practitioners and the farmers before embarking on vaccination and post-vaccination monitoring.

Training of staff, private veterinarians and farmers

A total of 118 staff and private veterinarians from 23 districts were trained in all aspects TBD control including the ECF immunisation technique to equip them with the skills necessary to handle the vaccine and carry out the post-immunisation monitoring. Twelve sensitisation meetings were also conducted for a total of 454 farmers from 13 districts.

ECF immunisation activities

Twenty-five immunisation programmes were carried out in 12 districts covering 138 farms during which 2005 animals were immunised. The results showed that 91% experienced mild to moderate reactions while mortality was recorded in 0.16% of the animals. The protection in vaccinated cattle was 86%.

Post-immunisation monitoring

Monitoring covers a period of 28 days after the vaccination and requires the participation of both the farmer and a veterinarian. The farmer takes the temperature daily and records it on charts. He/she is urged to report any abnormal behaviour to the veterinarian immediately. The veterinarians make about six visits to ensure that the temperature is being taken correctly, to reassure the farmer when the temperature starts rising, to take blood/lymph

smears and to provide treatment as required. The project staff make 2–3 visits to reassure and give advice to the veterinarian and the farmer. The laboratory staff examine slides and carry out the indirect fluorescent antibody test to determine serological status pre- and post-immunisation. Data analysis is carried out to determine the responses of the animals to vaccination.

Introduction of cost-recovery

For the vaccination programme to be sustainable, it was recommended that cost-recovery be introduced. Consequently, partial cost-recovery was introduced in two districts where farmers initiated introduction of the vaccine after being impressed by the results. Such farmers pay US\$ 5 per animal to be vaccinated. This goes towards the cost of drugs, transport and the staff welfare. Under this partial cost-recovery arrangement a total of 672 head of cattle have been vaccinated on 61 farms.

Tick control following immunisation

Modification of tick control method following introduction of TBD vaccination is an important incentive for introducing ECF immunisation to the farmer since he/she should be able to reduce his/her acaricide use and thus make a financial saving. Consequently, on-farm investigations were initiated on four farms where vaccination had been carried out and the farmers had been engaged in intensive tick control. The results showed that the farmers could reduce the dipping interval from twice a week to once a fortnight, depending on the type of acaricide used, farming system, environment and tick population. From this exercise, a 50–60% reduction in tick control using acaricide is recommended.

Setting up the infrastructure for vaccine delivery

To manage and run a country-wide TBD vaccination programme effectively, it was suggested that a co-ordinating unit be set up at Entebbe with an administrative office and a highly secured store for keeping drugs, chemicals, and equipment. The project has the support of the Technology Validation Committee on ECF immunisation that met twice and made favourable recommendations on its performance. A building has already been refurbished to serve both as an office and store for the project.

Future activities of the ECF immunisation programme

The ECF immunisation programme has stimulated much enthusiasm amongst the dairy farmers and many agree the programme is operating satisfactorily. Therefore, there is potential for expansion of the programme in the country.

The number of target cattle for ECF immunisation in Uganda is projected as follows:

Improved dairy cattle	
Present population	232,000
Primary emphasis on young stock: 2–18 months (25% of total)	58,000
Secondary group: 50% of adult cattle	86,000
Replacement stock per year (20,000) over 5 years	100,000
Total dairy cattle to be immunised over 5 years	244,000
Improved beef cattle	
Population of beef stock on ranches	200,000
Target calves 2–6 months	25,000
Replacement stock (20,000/year) for 5 years	100,000
Total beef cattle to be immunise over 5 years	125,000

The potential number of dairy and beef cattle to be immunised over a 5-year period is 369,000 head at an annual average of over 70,000 head.

ECF immunisation in Zambia

M. Mangani

Department of Veterinary Services, P.O. Box 50060, Lusaka, Zambia

Tick and tick-borne disease (TBD) control in Zambia is mainly orientated towards the prevention of East Coast fever (ECF). The activities are supported by the Belgian government under a programme called 'Assistance to the Veterinary Services of Zambia' (ASVEZA). This programme operates in Eastern (ASVEZA east) and Southern (ASVEZA south) Provinces. The information in this report is mainly obtained from the ASVEZA annual report for 1996. The separation of activities into ASVEZA east and ASVEZA south is due to differences in the epidemiology of the disease in the two Provinces; ECF is endemic in Eastern Province and epidemic in Southern Province.

ASVEZA East

Control

Control of ECF using the infection and treatment method of immunisation (ITM) began in the late 1980s. To date, approximately 120,000 calves have been immunised in this Province. Immunisation is based on the use of a local *T. parva* Katete isolate as vaccine. Immunisation was initiated in Chipata District and was then extended to neighbouring Districts in Eastern Province (Table 1).

Table 1. *Areas immunised in Eastern Province.*

District	Area
Chipata	Whole district
Katete	Southern part of Great East road (except Mtandaza) veterinary camps of Vuramboko, Kwenje
Chadiza	Whole district (except Sinalo crush pen)
Lundazi	Whole district (except Chikomeni, Magodi and Chama veterinary camps)
Petauke	Sinda and Nyangwe veterinary camps

Until 1995, immunisation was performed free of charge to the farmer. However, since 1996 cost-recovery for immunisation has been introduced, initially at a nominal charge of US\$ 15, which represents about 20% of the actual current cost of immunisation in Eastern Province. The amount charged will be steadily increased until the full cost of immunisation is covered. Before cost recovery was implemented, an extensive

information campaign was conducted in all districts to let the farmers appreciate the need for immunisation and allow them to decide on the strategy they wished to use for ECF control. Questionnaires collected from farmers during this information campaign showed that they were convinced of the need to control ECF and that, usually, failure to take up immunisation was due to lack of available money. Table 2 summarises the immunisations performed.

Table 2. *Summary of immunisations administered.*

Campaign	No. calves immunised	Overall seroconversion	<i>T. parva</i> stabilate used
Oct 1995–Jan 1996 (free of charge)	12,611	76%	V 13
July–Oct 1996 (with charge)	2,469	45%	V 16

Delivery

Since mid-1994, the Department of Animal Production and Health (DAPH) has been responsible for carrying out all immunisations. Under this arrangement, the District Veterinary Officers (DVOs) are in charge of the field implementation of campaigns and managing the funds, while the ASVEZA project provides logistical support and field equipment. The farmers cover the full cost of any theilericidal treatments which may arise and the money collected from the immunisations contributes to a revolving fund and is used to purchase oxytetracycline locally.

Trials on the viability of sporozoites on ice

If it were possible to transport and maintain thawed vaccine stabilate on ice, the cost of immunisation would be lower. Some trials were carried out in the laboratory to assess the threshold point of viability of sporozoites on ice. A field trial was also carried out in which 100 calves were immunised with stabilate, which had been thawed and kept on ice, while another 100 calves were immunised using the usual method. The experiment started in December 1996 and initial results are quite encouraging. Details will be made available as soon as a full evaluation has been completed.

ASVEZA South

In Southern Province, the government policy stipulates the use the local strains to control ECF by immunisation.

ECF stabilate production programme

T. parva Mandali stock

The infectivity of the Mandali stock available has been found to be very low for both cattle and ticks. Use of this stock has been discontinued pending further studies.

T. parva Chitongo stock

Chitongo was isolated in the mid-1980s from Chitongo village (Namwala District) and a bulk stabilate has since been produced. Preliminary titration trials have shown that high doses are required for immunisation, which indicates a low virulence. Chitongo may be considered as a mild stock and, if used as a vaccine, may not require the use of long-acting tetracycline. However, this mildness may be a disadvantage when producing stabilates for mass immunisation.

Cross-immunity trials

T. parva Chitongo versus *T. parva* Katete and reciprocal: breakthrough of *T. parva* Katete in a *T. parva* Chitongo immunised animal excluded the use of *T. parva* Katete in the Southern Province.

T. parva Chitongo versus *T. parva* trivalent vaccine: two trials have so far been carried out and a third experiment is under way to help refine the protection rate. Initial results are encouraging since animals immunised with *T. parva* Chitongo do not react when challenged with a lethal dose of *T. parva* trivalent vaccine. A field immunisation trial will begin on completion of the current experiment.

ECF control

The only viable alternative to immunisation in Southern Province is chemotherapy. Vector control in the traditional sector has been ineffective since acaricides and veterinary services are no longer free of charge and farmers are not yet accustomed to paying for them. The DAPH sells theilericidal drugs through ASVEZA on a full cost-recovery basis. The sales are organised through a network in the districts of Mazabuka, Choma, Kalomo, Monze and Gwembe. The system has improved the availability of theilericidal drugs in the Province allowing the farmer to choose which control option to use. The impact of this system is currently evaluated only through the amount of sales. The number of requests for treatment increased during the peak of the ECF season (Table 3).

Table 3. *Sale of theilericidal drugs during the period November 1995 to December 1996.*

Period	Turnover (ZK)	Days	Turnover/day (ZK)
Nov-1995	150,000	29	5,172
Dec 1995-Feb 1996	930,700	91	10,227
March 1996	170,000	23	7,391
April-May 1996	776,200	55	14,113
June-August 1996	4,157,300	88	51,635
Sept-Dec 1996	5,647,000	148	38,155

Immunisation against theileriosis in Zimbabwe

U. Ushewokunze-Obatolu

Veterinary Research Laboratory, P.O. Box CY551,
Causeway, Harare, Zimbabwe

Policy background

Agricultural framework

The agricultural sector is central to the economy, contributing on average, 23% of the gross domestic product and occupying nearly 70% of the population. The current agricultural policy, while supportive of the commercial sector, focuses on the smallholder producer and aims to address issues of poverty, malnutrition and low income by supporting agricultural production and the growth of a rural based agro-industry. Cattle are central to this sector, which contains about 67% of the national herd, and are dual purpose. They are also important for crop cultivation and transportation.

Control of ticks and tick-borne diseases

The integrated approach to the control of ticks and tick-borne diseases (TBDs) has been attractive to the Department of Veterinary Services for its biological advantages and its economic benefits. It has thus been an area of research for much of the last decade with financial assistance from Danish International Development Agency (DANIDA) and, in more recent years, from the Belgian Government through the Food and Agriculture Organization of the United Nations (FAO). This work resulted in the adoption of an approach based on a locally produced live vaccine, BOLVAC, which has now been registered for use without simultaneous tetracycline treatment.

The use of this vaccine must however, be complemented by concurrent coverage with vaccines against anaplasmosis and babesiosis (*Babesia bigemina*), both of which are produced by the department's laboratory. The use of a *B. bovis* vaccine, also produced by the laboratory, is being considered in particular risk areas.

The second component of the integrated approach entails the continued reduction of dipping. The dipping policy has shifted from an intensive system to a reduced regime that encourages the establishment of enzootic stability while maintaining effective tick control, and this has been promoted by changes in the regulations.

Control of theileriosis by immunisation

The approach to immunisation centred around the use of vaccines in problem herds. Veterinarians who have been trained over the last two years in various aspects of vaccine delivery and monitoring for theileriosis identify these herds. The Directorate must also agree for an identified herd to be immunised. Vaccination is then carried out in the dry season primarily to avoid the risk of exacerbating field exposure in the wet tick-active season and, secondly, to ascertain that immunity is due to the vaccine. Likewise, monitoring of the immunised herds by both clinical and serological means is mandatory. This is greatly assisted by the rapidly improving field disease monitoring system.

So far, 17,000 cattle have been immunised, 11,000 head on commercial properties and 6000 head in communal areas. Well over 13,000 cattle have been immunised over the last two years without the use of tetracycline, a feature which supports the agricultural policy through cost reduction. A charge of Zimbabwean Dollar (Z\$) 5.00 per head is levied which goes to a Departmental revolving fund.

The vaccine has been largely successful, with seroconversion in 93–100% of immunised animals. Its success is further confirmed by evidence of disease only in unvaccinated animals in some immunised herds.

Pending issues

Research

- An effective, safe and economical heartwater vaccine is anxiously awaited, particularly in view of changing heartwater epidemiology and the presence of the disease in areas where only theileriosis occurred previously.
- knowledge is needed to help describe the exact regimes for farmers to follow for tick control in various ecological zones.
- tests still need to be developed to help determine the impact of the vaccine and differentiate it from related field parasites.
- More information is required on the carrier state resulting from the vaccine and its implications for the epidemiology of theileriosis
- Studies should look into buffalo-derived theileriosis and its implications for the integrated control of TBDs.

Capacity and sustainability

- The cattle population at risk in theileriosis endemic zones in the five provinces (see map) is estimated to range between 15,000 and 20,000 head. There are questions about the Department's ability to meet an increasing demand for vaccine as currently, the basis for immunisation is highly selective and therefore coverage is low.

- At present, the department enjoys a core of trained staff with the required skills to produce the vaccines but a high staff turn-over rate may cause problems in staff capacity in the future.
- Much remains to be done to help ensure the Department's capability to meet the increasing demand. This needs to be supported by socio-economic and epidemiological studies to determine the viability of the immunisation service while continually looking

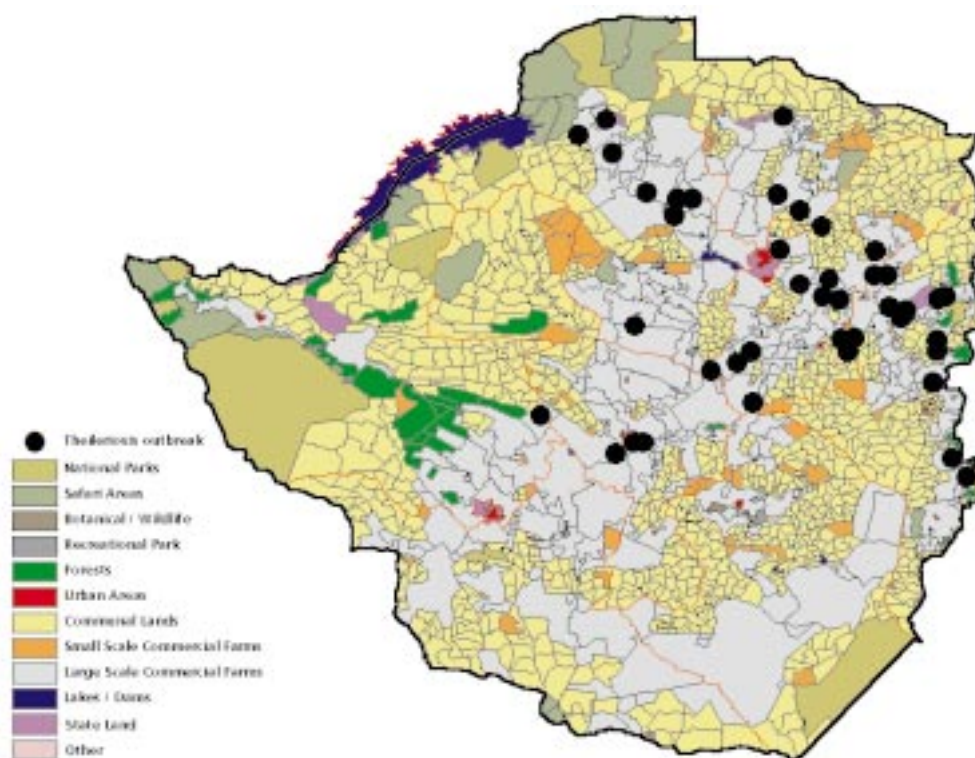


Figure 1. Theileriosis distribution in the five provinces – endemic zones – of Zimbabwe.

into possibilities for alternative production arrangements.

Tick-borne disease (TBD) immunisation in Swaziland

R. Thwala

Veterinary Services, P.O. Box 162, Mbabane, Swaziland

Introduction

Swaziland has a dual livestock production system composed of the communal sector, which is mostly on Swazi Nation Land (SNL) and a commercial sector, which is mostly on Title Deed Land (TDL). Swaziland has a cattle population of 641,979 and a goat and sheep population of 459,362 (Ministry of Agriculture and Co-operatives, 1995 livestock census). Around 77% of the cattle are produced in the communal sector and about 21% in the commercial sector. The dairy herd of 8184 animals is mostly composed of Friesian/Holstein and Jersey breeds and approximately equal numbers of dairy animals are found in the communal and commercial sectors.

Background

Recent studies by the Ministry of Agriculture and Co-operatives and the Food and Agriculture Organization of the United Nations (FAO) staff in 1995/96 have revealed that the significant tick-borne diseases (TBDs) in Swaziland, in order of importance, are babesiosis (Asiatic redwater), caused by *Babesia bovis*, heartwater caused by *Cowdria ruminantium*, and anaplasmosis caused by *Anaplasma marginale*. Since the introduction of *Babesia bovis* into Swaziland in the late 1970s, there have been epidemics of the disease on both SNL and TDL sector farms. Most cattle on SNL are indigenous Nguni crossed with Brahman, Simmental, Friesian or Jersey breeds. In these cattle, indications are that outbreaks are usually severe at first introduction of the disease after which only a small proportion of the population is affected, according to tick population dynamics. In the TDL sector, where dipping is more stringent and effective, the immunity of the herds to all four TBDs is low and outbreaks, although not common, can be very severe. From serological survey results and disease outbreak reports, heartwater and babesiosis due to *Babesia bigemina* (African redwater) seem to be more prevalent in the communal sector, although deaths from these diseases are negligible compared to those from Asiatic redwater and, to a lesser extent, anaplasmosis.

Goats in both SNL and TDL farms are highly susceptible to heartwater and morbidity and mortality rates may be high, especially in exotic breeds.

Disease diagnosis at the Central Veterinary Laboratory indicates that TBDs constitute between 20% and 40% of the total number of cases of disease diagnosed in cattle. Although

this may be a biased conclusion, diagnoses made in the field give a similar picture. The economic losses incurred through death, loss of production and drug costs, as a result of TBDs have yet to be assessed.

Tick-borne disease immunisation

Field trials using TBD vaccines in Swaziland were started in 1990 by Dr Gavin Ramsay who used Australian *Babesia bovis*, *Babesia bigemina* and *Anaplasma centrale* vaccines on over 200 cattle of various ages on one of the government cattle breeding stations (GBS) in the highveld. The objective was to test a TBD control strategy for Swaziland based on the lessons learned from the vaccination and strategic dipping. The results were promising with 100% seroconversion to all three parasites after immunisation and no clinical losses. However, the trial was discontinued due to lack of funding.

In 1995, the government of Swaziland, with the assistance of FAO, initiated the immunisation of cattle against TBDs. The main objective was to assist the Department of Veterinary Services in the development of an integrated tick and TBD control strategy that could be tested and adapted nationally. Such a strategy would need to be both environmentally friendly and economically and scientifically sustainable. Work began on four government breeding stations and two private ranches. Calves below 12 months of age were given *Cowdria ruminantium* (Ball 3 strain), *Anaplasma centrale*, *B. bovis* and *B. bigemina* blood vaccines from Onderstepoort Veterinary Institute (OVI). These trials later included adult animals and threshold dipping within the herds. Studies were extended to immunise animals on a voluntary basis in communal areas (SNL) starting at two dip tanks. Six SNL pilot sites were then selected from the different physiographic zones. Currently, 700 animals (mostly calves aged below 12 months) on GBS, and 500 calves with 160 adults on SNL have been immunised with all four vaccines.

Heifers vaccinated on GBS have been put under a threshold tick management programme. To date, the results from the 1996 study have been encouraging. At the highveld ranch site, for example, vaccinated animals were dipped four times during the year instead of the usual 30 or more dippings carried out on the unvaccinated herd. During the same period, five confirmed cases of TBD occurred in the unvaccinated animals while the vaccinated group registered none. This should be interpreted with caution as continued dipping of unvaccinated animals meant that they were acting as 'sweepers' of ticks thus, leading to a reduced TBD challenge. However, once all the cattle are under threshold dipping a more conclusive result can be expected. It is vital that this work be continuously monitored and extended to other sites.

Conclusion

The trials conducted so far have revealed some very promising results in relation to the project objectives. Therefore, it is likely that the commercial sector will be willing to adopt this integrated strategy once the relevant legislation has been reviewed to make this possible.

The trials should also be extended to the SNL to determine the most appropriate management and delivery system before the strategy can be implemented on a national scale. As the project funding came to an end in December 1996, there is a danger that this important work may suffer another serious blow if funding is not found in time to address the outstanding issues for a sustainable national programme to be put in place.

TBD immunisation in South Africa

D. De Waal

ARC Onderstepoort Veterinary Institute, Private Bag X05,
Onderstepoort 0110, South Africa

Summary

In South Africa (SA), three tick-borne diseases (TBDs) occur which are of great economic importance to the livestock industry in the country. They are babesiosis, anaplasmosis and heartwater (cowdriosis). East Coast fever (ECF), caused by *Theileria parva*, was introduced into the country at the turn of the century. Fortunately, however, an intensive eradication campaign over a 50-year period finally eliminated the disease in 1954 at a cost of 100 million South African Rands (R)(US\$ 22 million).

At present, more than 90% of the total cattle population in the country occurs within the known *Boophilus* spp distribution area and is therefore potentially at risk to babesiosis and anaplasmosis. Nearly 38% of the cattle, 8% of the sheep and 5% of the goat populations occur in the *Amblyomma* distribution area. Official veterinary reports indicate that TBDs contribute for more than 19% of total annual livestock losses. TBDs cost SA in the region of R 400 million (US\$ 88 million) per annum, 20% of which is the cost of acaricides alone, while 40% is due to production losses.

Four methods of control (or combinations thereof) are generally applied to fight TBDs in SA. These include specific treatment of clinical cases, tick control, chemoprophylaxis and vaccination. Control of these diseases by intensive tick control alone, is not a solution to the problem in areas where vectors are well established and is generally not recommended. The recommended long-term approach to managing ticks and TBDs in SA is integration of the strategic use of acaricides to allow natural exposure of young animals (calves/lambs/kids) to TBDs when they are naturally resistant to or protected by passively-acquired maternal antibodies, with vaccination. This compensates for epidemiological factors that may inhibit the exposure of these animals to infected ticks when young.

The use of vaccines to protect animals against TBDs is not new to SA and vaccines against *Babesia bigemina* and *Anaplasma* have been produced since 1912, with *B. bovis* being included in 1953. Early attempts at the turn of the century to vaccinate animals against heartwater were less successful as most animals died of vaccination reactions, which could not then be controlled. Neitz and Alexander used the innate resistance of young animals to vaccinate them against heartwater. However, the discovery of chemotherapeutic drugs with which heartwater reactions could be treated in the 1940s, made it possible to extend vaccination to older animals.

Since the early 1980s, attenuated strains of *B. bovis* (S-strain) and *B. bigemina* (G-strain) have been used to vaccinate cattle against babesiosis. Both monovalent frozen and bivalent unfrozen (chilled) *Babesia* vaccines are routinely available and approximately 200,000 doses

of vaccine are sold per annum. The original isolate of *Anaplasma centrale*, identified and used by Theiler as a vaccine for *A. marginale*, is still used to produce the live blood vaccine today. Both a frozen and an unfrozen (chilled) *Anaplasma* vaccine is produced and approximately 220,000 doses are sold per annum. The Ball-3 isolate of *Cowdria ruminantium* is used for producing the heartwater vaccine (frozen only) and approximately 120,000 doses are sold per annum.

Each batch of blood vaccine has to pass a number of strict quality control checks before it is issued. These include freedom from bacterial and fungal contamination, absence of adventitious agents and innocuity. Strict measures are also taken to prevent the inadvertent spread of other blood-borne infections with the blood vaccine. Potency tests are also performed on all the frozen vaccines.

Ever since these vaccines started being used, outbreaks of disease have occasionally been reported in previously vaccinated animals. Many factors may play a role in the occurrence of the disease, such as storage failures, mishandling of the vaccine, incorrect administration, incorrect diagnosis of the cause of the outbreak, treatment of animals with sterilising drugs during or after vaccination and rigorous tick control following vaccination. Insufficient cross-immunity between vaccine strains and certain field strains is known to occur in *A. centrale* and *C. ruminantium*. However, laboratory investigations indicate that antigenic/strain variation in the case of *Babesia* probably plays only a minor role, if any, in disease outbreaks in previously vaccinated cattle in SA.

Chemoprophylaxis, as a method of short-term control, is often used in SA, particularly in situations where the temporary residence of susceptible animals is in an infected area (e.g. agricultural shows), when pregnant cows are at risk and in the control of disease outbreaks. In babesiosis, imidocarb is most commonly used; however, no chemoprophylactic drugs are available for the control of anaplasmosis and heartwater. The chemoprophylaxis of both anaplasmosis and heartwater is, therefore, based on the repeated administration of chemotherapeutic drugs (tetracyclines) to control parasitaemias in naturally infected animals.

Vaccine production

Production of *Theileria parva* stabilates for immunisation against theileriosis

F. Musisi

Food and Agriculture Organization of the United Nations (FAO) Office,
P.O. Box 30750, Lilongwe 3, Malawi

Introduction

Tick-borne diseases (TBDs), especially East Coast fever (ECF), have traditionally been controlled by dipping or spraying cattle. However, due to the increasing cost of acaricides and an increased awareness of their impact on the environment, alternative methods are being sought. The search for a vaccine against ECF began as early as 1914 (Spreull). However, practical means of immunising against ECF were only developed in the 1970s. Even then, application of the technique on a large scale did not begin until the mid-1980s.

Immunisation is a useful way of reducing reliance on acaricides to control tick-borne diseases. ECF vaccine production in Malawi was set up in conjunction with production of other TBD vaccines at the Tick-borne Diseases Vaccine Production Centre. Construction of the Vaccine Production Centre (VPC) in Lilongwe started towards the end of the 1980s. The government of Malawi set up the VPC with the technical assistance of the Food and Agriculture Organization of the United Nations (FAO) and financial co-operation of various donors, principally the governments of Denmark and the Netherlands and United Nations Development Programme (UNDP). The objectives were to look into the available technologies for immunisation against TBDs and to subsequently evolve production systems that would meet the minimum standards of registerable vaccines under Good Manufacturing Practices (GMP).

This presentation outlines the salient features of the premises, the methods of production and the quality control mechanism instituted for the manufacture of *Theileria parva* stabilates for immunisation. It describes how these aspects were managed at the VPC before production activities were suspended.

Premises for vaccine manufacture and organisation of technical management

During the development phase, the four TBD vaccines were managed on a project basis according to the technology involved in production. Thus the ECF vaccine was developed as the ECF project based on tick culture technology, while the *Anaplasma*, *Babesia* and heartwater vaccines were handled within a separate project since all three are based on the

utilisation of blood from splenectomised animals. During this phase, the technologies and facilities required for vaccine production were developed.

It was decided that the two product-development projects had been sufficiently successful to warrant advancing all four vaccines to a production phase.

Premises

Purpose-built facilities were developed for the manufacture and technical servicing of the TBD vaccines as follows:

- A farm comprising an animal quarantine station, fields for growing feed crops and grass for silage and a tick-free, multi-unit housing facility for up to 300 cattle.
- An animal isolation compound with tick-proof pens for holding infected cattle.
- An isolation unit for holding up to 160 rabbits.
- A vaccine manufacturing laboratory with separate wings for production (Figure 1) and quality control (Figure 2).
- A separate building for veterinary advisory work, including the handling of field samples and training (Figure 3).
- A hostel with accommodation for up to 16 trainees and scientists from countries in the region.

Responsibilities

The Technical Manager had overall responsibility for technical aspects of the VPC with three technical departments, each reporting independently to him/her. The departmental distribution of responsibilities was as follows:

Production

- Production of all vaccines, namely, ECF, *Anaplasma*, *Babesia*, heartwater.
- Production of antigens and reagents for serology and diagnosis.
- Maintenance of the vaccine seed banks.
- Cleaning and sterilising all glassware used in Production and Quality Assurance departments
- Preparation of media.
- Repair and maintenance of vaccine production equipment.

Quality Assurance (QA) and Product Improvement

- Quality control (QC) of the seed bank.
- Quality control of all raw materials including donor animals.

- In-process control testing during the manufacture of all vaccines.
- Quality control testing of the final products (vaccines, serological antigens and reagents) including commissioning of external testing and QC certification.
- Good manufacturing practice (GMP) /QA monitoring of the vaccine facilities including validation of processes and monitoring of good laboratory practice (GLP) and safety in the laboratories.
- Documentation including batch records, Standard Operating Procedures (SOP) and Manufacturing Instructions (MI) for all vaccines.
- Serological and microscopical diagnostic support to the Veterinary Advisory Department.
- Co-ordination of the Product Improvement and Cost Reduction Programme.

Veterinary Advisory Service

- Farm and experimental animal facilities.
- Selection and acquisition of all animals.
- Performance and co-ordination of field trials.
- Product complaint register analysis.
- Isolation and characterisation of field parasites associated with possible vaccine breakthrough.
- Support for making differential diagnosis.
- Co-ordination of training.
- Conducting experiments using target animal species in support of the Product Improvement and Cost Reduction Programme.

Short-term priority areas for the Product Improvement and Cost Reduction Programme

In-house activity

- *In vitro* quantification of the immunising parasite.
- Determination of the immunising and/or field dose.
- Preparation and standardisation of challenge stocks for the vaccines.
- Optimisation of batch size.
- Vaccine stock development for ECF vaccines.

External research support

- Antigenic analysis and characterisation of field isolates of epidemiological significance.

- *In vitro* quantification of the immunogenic forms of the parasites and/or the immunising antigen.
- Enzyme-linked immunosorbent assay (ELISA) technology for QC of TBD vaccines.
- Vaccine stabilisation, preservation, storage and minimisation of dependence on the liquid nitrogen chain.

Description of trivalent *T. Parva* vaccine stabilate

This tick-derived stabilate consists of sporozoites of three *T. parva* stocks: cattle-derived Muguga, cattle-derived Kiambu 5 and buffalo-derived Serengeti-transformed. Each is harvested in Minimum Essential Medium containing bovine plasma albumin (MEM/BPA) and glycerol, and stored frozen in liquid nitrogen. The stabilates are supplied in 2 ml vials containing 5, 10, or 20 doses. Radley (1981) noted that a pool of these parasite stocks protected cattle against challenge with stocks from Kenya, Malawi, Tanzania and Uganda.

Inoculation of the trivalent stabilate and simultaneous administration of an appropriate tetracycline allows these three stocks to establish in the animal. The animal develops immunity to immunologically related *T. parva* infections. Immunity is usually fully developed within four weeks of immunisation.

Production process

The essential features of the production process are summarised in Figure 4. Most of the steps are common to the routine laboratory production of *T. parva* stabilates. However, the process differed from routine stabilate preparation described by Cunningham et al (1973) in the following ways:

1. Stabilates of individual component stocks were mainly cryopreserved in transfusion bags (Njuguna and Musisi 1996) with only 60–80 ml of stabilate being dispensed and cryopreserved in 2 ml cryoampoules. Both transfusion bags and cryoampoules were kept at -70°C .
2. Viability and appropriate mixing ratios of component parasite stocks were determined on the basis of prepatent periods to parasite and fever detection in three pairs of cattle using cryoampoule-preserved stabilate. Usually the mixing ratios were 1:1:2 or 1:1:3 (Muguga: Serengeti-transformed: Kiambu 5).
3. Based on the mixing ratios, a sample pool of the trivalent stabilate was prepared using stabilate from transfusion bags for each parasite stock. The pool was then diluted and titrated at twofold dilutions starting from undiluted through to 210 using 4 cattle per dilution.
4. A practical immunising dose was worked out based on sample pool titration results in combination with Karber's (1931) method to determine the ID_{50} .

5. The actual vaccine pool was prepared using stabilate in transfusion bags for each parasite stock by mixing them in the predetermined proportions. To obtain 5-, 10- and 20-dose packs, the vaccine pool received an appropriate amount of stabilate diluent after which the vaccine was dispensed.

Outline of quality control methods

Selection of cattle for vaccine production

Historically, cattle used for ECF vaccine production originated from the southern region of Malawi where no East Coast fever has been recorded. Furthermore, the farms from which such cattle originated practised a strict tick control regime. When brought in, the cattle were quarantined for 7 days before being transferred to the VPC farm. None of the cattle obtained from outside the VPC farm were used for vaccine production until a minimum period of 8 weeks of clinical monitoring had been completed. More recently, virtually all bovine used in the production (but not testing) of the vaccines were produced on the VPC farm.

The cattle used were confirmed to be free from and serologically negative to *Anaplasma* spp, *Babesia* spp, *Theileria parva* and *Theileria mutans*. Taurine breeds, particularly Friesians, and their crosses were used for immunisation. Normally, the animals were between 9 months to 2 years of age and weighed a minimum of 120 kg. The cattle were fed silage and concentrates; no hay or fodder was given to the animals.

The TBD farm is double fenced and human and animal traffic is restricted.

Sterility testing

Special attention was paid to checking the sterility of the following samples:

- Media for stabilate preparation.
- Surface-sterilised *T. parva*-infected ticks before grinding.
- Ground-up tick supernate, at the start of dispensing, mid-dispensing and at the end of dispensing.
- Pooled components making up the ultimate vaccine as described above.

All the samples collected were tested for bacterial contamination as described in the Production manual. Essentially this involved incubating samples in Fluid Thioglycollate Medium (FTM) or Soyabean Casein Digest Medium. Upon detecting growth, identification of the organism was undertaken as described in the manual.

Viability testing

Stabilates of the three stocks in the trivalent *T. parva* stabilate vaccine were prepared separately. The viability of each stabilate was assessed using a twofold dilution in cattle as described in the Vaccine Production manual. Assuming the stabilates were infective, the

results obtained were used to determine the appropriate mixing ratios for the 3 stock stabilates.

Potency testing

The potency of the vaccine was initially assessed by determining the ID₅₀ and PD₅₀ of a sample vaccine pool, made on the basis of the viability and mixing ratios as described above. This was followed by inoculation of small groups of cattle to determine a practical immunising vaccine dose. Finally, potency of the final vaccine stabilate pool was confirmed by inoculation of the working vaccine dose and twice the working dose as determined using the sample vaccine pool. Using double the recommended vaccine dose helped provide data on the safety of the vaccine in cattle.

Freedom from contaminating viruses

To confirm freedom from Enzootic Bovine Leucosis (EBL) and Bovine Viral Diarrhoea (BVD) viruses, 20ID₅₀ ECF vaccine doses were injected into pairs of cattle known to be free from EBL and BVD viruses. The serology of these cattle was monitored for evidence of EBL and BVD infection on days 0, 14, 21, 28, 35, and 42 after injection. Concurrently, the development of schizonts and fever were monitored. When the cattle became clinically ill, they were treated with anti-theilerial drugs to allow their survival for the detection of EBL and BVD virus contamination in the vaccine.

Innocity and safety

To help confirm safety of the vaccine stabilate batch prepared, guinea pigs and suckling mice were inoculated with 0.5 ml and 0.1 ml, respectively, of freshly reconstituted trivalent *T. parva* stabilate. A pair of uninoculated guinea pigs and 4 suckling mice were kept as controls together with the inoculated animals. Essentially, if all the inoculated animals survived three weeks without any clinical disease or death, the vaccine was considered safe for use.

Other considerations

Effect of freeze-thaw of the vaccine

Whilst, during preparation of the trivalent *T. parva* stabilate vaccine, it is possible to thaw, pool and refreeze the stocks under controlled conditions, this must not be done with vaccine in the field. Freeze-thaw of the vaccine under field conditions would inactivate it completely; thus any unused thawed vaccine must be discarded. It is recognised that, even under controlled laboratory conditions, there is a limited loss of viability. But, this is considered necessary to make the trivalent *T. parva* stabilate user-friendly. If pooling was left to field personnel with varying competency in laboratory techniques, there would be a risk of over- or under-dosing with vaccine.

Stability of thawed vaccine

Experiments, in which the vaccine was thawed and subsequently maintained on ice, show that the vaccine remains viable for up to 18 hours (Musisi et al 1996). However, for routine field use, the vaccine should be thawed and maintained on ice for no more than 8 hours (i.e. a working day). This is because repeated opening and closing of the vaccine container affects the pH resulting in loss of viability of *T. parva* sporozoites.

Diluent packaging

Diluent was prepared for the different vaccine dose packs. It was sterilised, dispensed and sealed in such a manner that the pH remained stable. Stabilate was transferred from the cryoampoule to the diluent vial with a syringe and needle. This approach eliminated potential mistakes that could be made by vaccinators attempting to implement their own dilutions.

Conclusion

The above description outlines the minimum acceptable standards regarding the premises, organisation and methodology for production of *T. parva* stabilates for wide-scale field immunisation. Efforts at the VPC took the process from a routine laboratory approach towards professional and Good Manufacturing Practices. The VPC management recognised that further research was needed to help make the products user-friendly and to investigate various aspects, such as how to avoid losses from thawing and re-freezing during production; safety of the vaccines in different breeds and during pregnancy; minimum age to immunise and post-immunisation reactions. These issues were being addressed at the time that VPC operations were suspended and, therefore, require attention when vaccine production is resumed.

References

- Cunningham M.P., Brown C.G.D., BurrIDGE M.J., Joyner L.P. and Purnell R.E. 1973. Cryopreservation of infective particles of *Theileria parva*. *International Journal for Parasitology* 3:89–95.
- Karber G. 1931. Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. *Arch. Exp. Pathol. Pharm.* 162:480.
- Musisi F.L., Quiroga J.C., Njuguna L.M., Kamwendo S.P. and Chamabala K.E. 1996. Viability of *Theileria parva* trivalent stabilate after thawing and maintenance at 40°C for up to 15 hours. *International Journal for Parasitology* 26:175–179.
- Njuguna L.M. and Musisi F.L. 1996. Bulk-freezing of *Theileria parva* stabilates for vaccine production. *International Journal for Parasitology* 26:67–70.
- Radley D.E. 1981. Infection and treatment method of immunization against theileriosis. In: Irvin A.D., Cunningham M.P., and Young A.S. (eds), *Advances in the Control of Theileriosis. Proceedings of an International Conference held at the International Laboratory on Animal Diseases, Nairobi, Kenya, 9–13 February 1981*. Current Topics in Veterinary Medicine and Animal Science Volume 14. Martinus Nijhoff, The Hague, the Netherlands. pp. 227–237.

Spreull J. 1914. East Coast fever inoculation in the Transkeian Territories, South Africa. *Journal of Comparative Pathology and Therapeutics* 27:299–304.

Toilet		
		Uninfected
Media		
Glassware		Office
Blood vaccines production laboratory		Tick
Blood processing dispensing		Tick
		Glycerolisation dispensing
Vaccine		
Deep freezers (–70°C storage)		–196°C Liquid nitrogen vaccine under quarantine

Figure 1. Production Department.

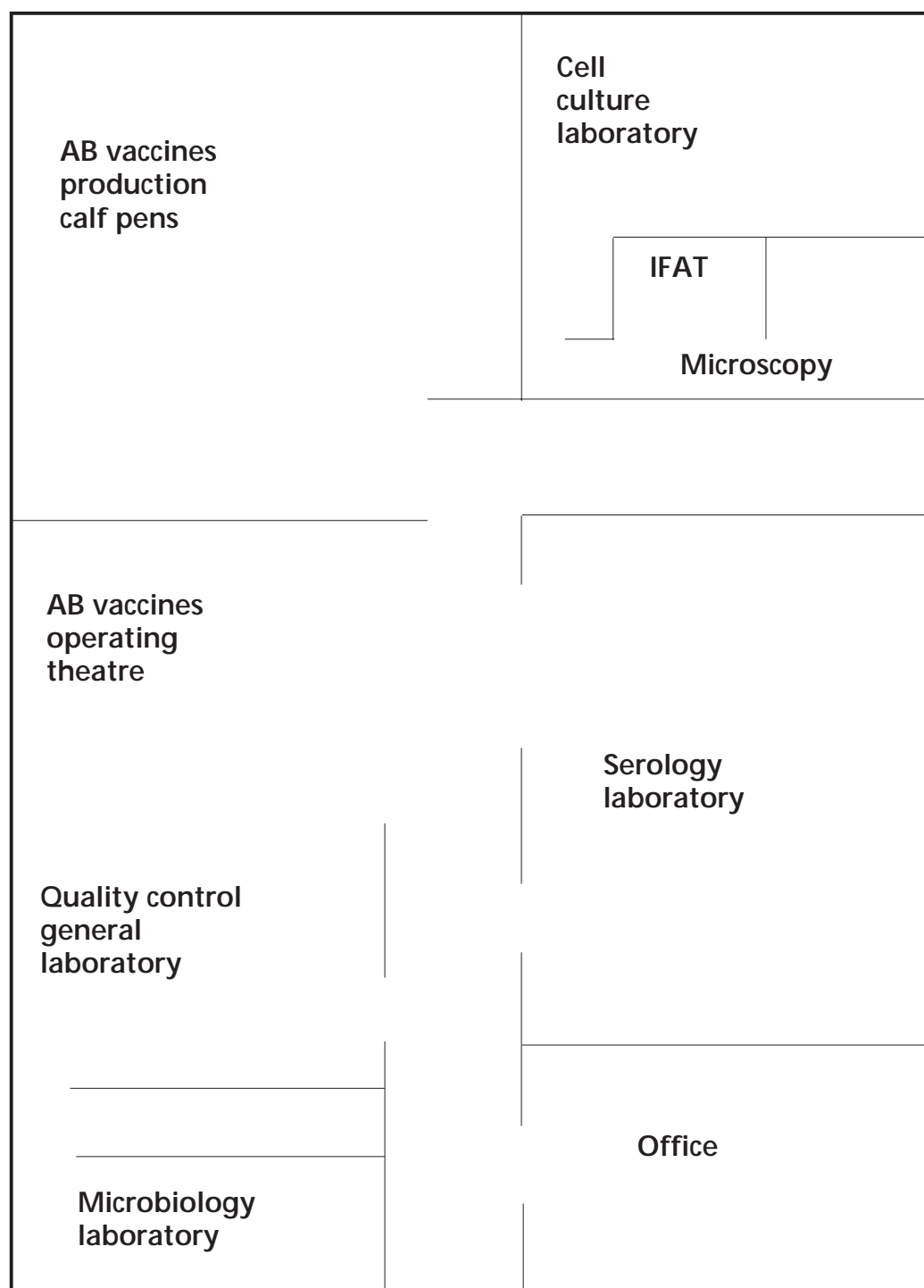


Figure 2. Quality Assurance and Production Improvement Department.

Toilet		Reception
Sales		General
Vaccine		Accounts
Veterinary advisory		Training
Secretary		
Field ticks		
Rabbits		Glassware wash
IFAT-field		Toilet
Store		

Figure 3. Management and Advisory Service.

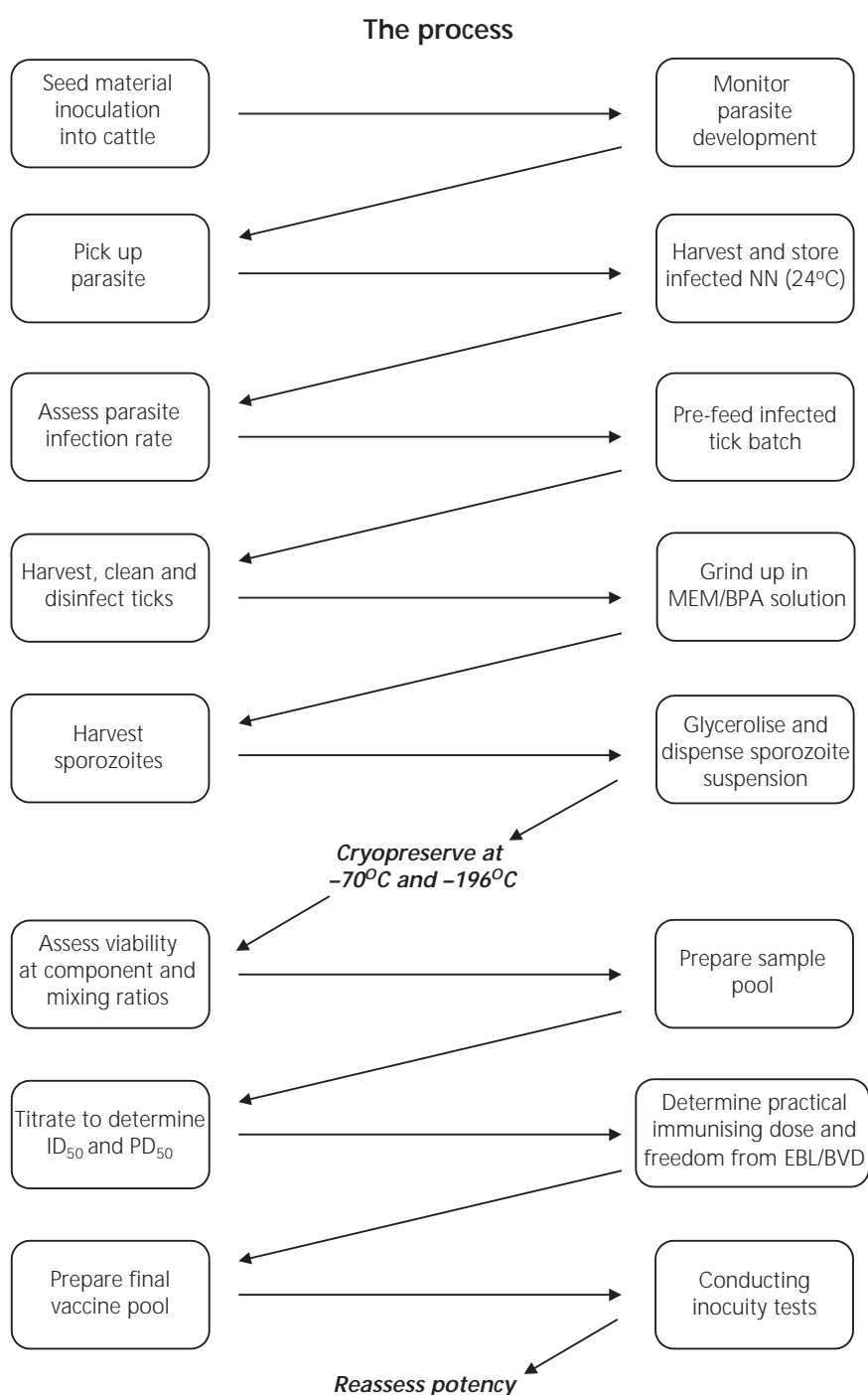


Figure 4. The process of production of a *Theileria parva* vaccine.

The preparation of a composite stabilate for immunisation against East Coast fever

S. Morzaria, P. Spooner, R. Bishop and S. Mwaure

International Livestock Research Institute (ILRI),
P.O. Box 30709, Nairobi, Kenya

Background

The preparation of *Theileria parva* sporozoite stabilates has been described by Cunningham et al (1973). The method is detailed in the FAO report (1978) and the FAO Tick and Tick-borne Disease Manual (1984). *Theileria parva* stabilates are a source of viable sporozoites that can be used to immunise cattle by 'infection and treatment'. This method involves inoculating cattle with a potentially lethal dose of sporozoites and simultaneous treatment with an oxytetracycline formulation to control the severity of the disease (reviewed by Radley 1981). After the development of the infection and treatment method of immunisation in the mid-1970s, an immunising stabilate referred to as the 'Muguga cocktail', comprising three stocks of *T. parva*—Muguga, Kiambu 5 and Serengeti-transformed — was prepared. This pool of stocks was selected because it gave broader protection than the individual immunising components in cattle cross-immunity trials (Radley et al 1975a; Radley et al 1975b).

The International Livestock Research Institute (ILRI) was requested by the Food and Agriculture Organization of the United Nations (FAO) to prepare a batch of the 'Muguga cocktail'. This was because a shortage of the immunising stabilate was predicted due to increasing demand and the Vaccine Production Centre (VPC) in Malawi not functioning at full capacity to meet this demand. ILRI agreed to prepare the vaccine stabilate since, at the time of the request, no other institution within the region was able to produce the 'cocktail'. Following consultations with FAO, the technical staff at VPC in Malawi and a number of other scientists working in the field of theileriosis, a protocol for the preparation of a composite stabilate comprising the three stocks was developed. A number of modifications from the original VPC protocol were introduced, some of which have been adopted as part of the standard ILRI procedure for stabilate preparation. Other amendments were also introduced to fulfil the requirements of the Office International des Epizooties (OIE) recommended standards for biological products (OIE 1996) and the proposed new standards that have been set by the Standards Committee, representing the Organization of African Unity (OAU), FAO and ILRI. The detailed protocol for the preparation and the *in vitro* and *in vivo* characterisations of the composite stabilate, designated FAO 1, are described below.

Protocol for a composite stabilate

Laboratories producing *T. parva* stabilates have made various modifications to the recommended protocol (FAO 1984). There have been variations in media used, containers for cryopreservation, methods of tick grinding and equilibration periods. More recently, freeze-thaw-freeze steps have been introduced to combine the individual components of 'cocktail' stabilates, before release for use.

After examining various options for the preparation of the cocktail stabilate, a modified protocol was formulated. This protocol was circulated for comments to various scientists involved in the preparation of vaccine stabilates. These included scientists from VPC, Malawi; National Veterinary Research Centre, Kenya; Onderstepoort Veterinary Institute, South Africa; Centre for Tropical Veterinary Medicine, UK; and the University of Utrecht, The Netherlands. Subsequently, a clearer protocol was developed in a detailed step-wise form with time-bound objectives for each step. The protocol took into account the quality control procedures and general standards recommended by OIE (1996), and those drafted by the OAU/FAO/ILRI Standards Committee (OAU/FAO/ILRI 1996). Special attention was given to both 'Good Manufacturing Practice', for the tick unit, animals, tissue culture media, serology, contamination with extraneous organisms, and for *in vitro* and *in vivo* methods of characterisation. In the protocol, reference was also made to the standards applied at each stage of preparation. The final protocol was approved by the members of the Programme Steering Committee of the Regional Tick and Tick-borne Diseases Control Programme and the FAO.

The significant changes to previous methods for preparation of 'Muguga cocktail' stabilates were:

1. The stabilate was prepared from pooled ticks so that equal numbers of infected acini were present from each component stock, Muguga, Kiambu 5 and Serengeti-transformed.
2. No freeze-thaw-freeze steps were required to mix component stocks.
3. The ticks used dropped from cattle during a range of low to high piroplasm parasitaemia.
4. *Theileria* infection rates in ticks were critically assessed.
5. *In vitro* characterisation was carried out, beginning with the seed stock and at various stages during the production to confirm the stability and integrity of the component parasite stocks.
6. The stabilate was prepared as concentrated as possible (the highest number of infected acini per ml) given the restrictions outlined in point 3.
7. Plastic straws of 0.5 ml capacity were used.

Stabilate preparation

A diagrammatic representation of the procedure for stabilate preparation is given in Figure 1.

During preparation of the composite stabilate, parasite isolations in tissue culture were made from cattle infected with the individual seed stocks, from *in vitro* infections using the

composite stabilate and from cattle infected with the composite stabilate. Again, these isolates were subjected to *in vitro* characterisation using a panel of monoclonal antibodies (MAb) and a set of DNA probes. The MAb and DNA profiles of these isolates were compared with those obtained from earlier isolates of these seed stocks. The MAb profiles of isolates, taken as biopsies from cattle infected with the three individual seed stocks, were similar to those obtained previously and confirmed the similarity between the Muguga and Serengeti-transformed stocks. The profile of the Kiambu 5 stock was similar to profiles of earlier isolates of this stock, but significantly different from the Muguga and Serengeti-transformed stocks.

Profiles from *in vitro* infections, using reference stabilates prepared from ticks which fed on cattle infected with the individual stocks, were also similar to the parent stabilates. These ticks were then pooled together to prepare the composite stabilate which, when used in *in vitro* and *in vivo* studies, gave isolates with profiles consistent with a 'pooled' profile of the three seed stocks. These findings, combined with those above, demonstrating the integrity of the individual seed stabilates, indicated that each seed component was present in the composite stabilate, although profiles of the Muguga and Serengeti-transformed stocks would be similar. Similarly, using the four DNA-based probes, the TpR, telomeric, LA6 and minisatellite sequences, the stability of all the three components was maintained throughout the preparation of the stabilate. The final stabilate, referred to as FAO 1, was first used to test the infectivity in susceptible cattle and secondly serial dilutions were used to immunise cattle using the infection and treatment method. After immunisation, recovered cattle were challenged with a lethal dose of each of the seed stocks. All animals that were immunised down to a 1:80 dilution, the highest dilution tested in this initial titration of the stabilate, were protected. Thus, both the *in vitro* and *in vivo* characterisations showed that the integrity of all the components of the cocktail was maintained in the FAO 1 composite stabilate.

Time-frames

The timeframe for the preparation of a vaccine stabilate depends on several factors, most importantly on whether the stock to be used is already fully characterised or is a new isolate. A number of important steps need to be taken for a new isolate to be considered a potential candidate vaccine stock. These include the preparation of a reference stabilate from the isolate, preparation of a working stabilate, *in vitro* characterisation, *in vivo* cross-immunity tests and field immunisation trials. This could take a variable period of up to 24 months. Assuming that satisfactory results are obtained, a decision can then be made to use the isolate to make a large vaccine stabilate. This preparation can take an additional 5–7 months, followed by laboratory titration in cattle requiring an additional 6–8 months. For FAO 1 the preparation of the large stabilate took 10 months, which included the testing of the seed stabilates, a preliminary titration in cattle and a comprehensive *in vitro* characterisation of the composite stabilate. Full *in vivo* characterisation, including the three-stage titration, will require an additional 6–8 months.

Since many isolates contain mixed *T. parva* populations, it is important that a vaccine stabilate is made using characterised seed (working) stabilate(s). This vaccine seed stabilate should be made in sufficient quantity so that it can be used for all subsequent stabilate preparations. Otherwise, product variability may be encountered which might affect the efficacy of the vaccine batches.

Summary and conclusions

- A large composite stabilate (FAO 1), of 10,000×0.5 ml straws, containing the components of the Muguga cocktail was produced.
- A modified method of preparing bulk composite stabilates was developed, applying the recommended 'standards'. The method may be considered suitable for other stabilates requiring single or mixed parasite stocks.
- In a preliminary titration, the stabilate successfully immunised exotic cattle, by infection and treatment, at a 1:80 dilution. These cattle were protected against a simultaneous, potentially lethal challenge of the three parent stocks included in the bulk stabilate.
- The full economic production costs (1996) are in the region of US\$ 120,000 (30–60 cents/dose).
- The total time taken to prepare the stabilate was 10 months.
- The FAO 1 stabilate requires a comprehensive titration in cattle using a three-stage design to identify a safe and efficacious dose.
- The total time, using characterised seed stocks, from cattle quarantine to completed titration is estimated at 16–18 months.

References

- Cunningham M.P., Brown C.G.D., BurrIDGE M.J. and Purnell R.E. 1973. Cryopreservation of infective particles of *Theileria parva*. *International Journal for Parasitology* 3:583–587.
- FAO (Food and Agriculture Organization of the United Nations). 1978. *Research on Tick-borne Diseases and Tick Control. Kenya, Tanzania and Uganda. Manual of Tissue Culture Techniques*. FAO Technical Report 3 (AG:DP/RAF/67/077). FAO, Rome, Italy. pp. 1–79.
- FAO (Food and Agriculture Organization of the United Nations). 1984. Immunisation against *Theileria parva*. In: *Tick and Tick-borne Diseases Control: A Practical Field Manual*. Vol. II. FAO, Rome, Italy. pp. 457–507.
- OAU/FAO/ILRI Regional Committee. 1996. *Standards for Live Tick-borne Disease Vaccines*. OAU/FAO/ILRI (Organization of African Unity/Food and Agriculture Organization of the United Nations/International Livestock Research Institute), Nairobi, Kenya. Draft.
- OIE (Office International des Epizooties). 1996. Theileriosis. In: *Manual of Standards for Diagnostic Tests and Vaccines*. OIE, Paris, France. pp. 321–330.
- Radley D.E. 1981. Infection and treatment method of immunization against theileriosis. In: Irvin A.D., Cunningham M.P., and Young A.S. (eds), *Advances in the Control of Theileriosis. Proceedings of*

- an International Conference held at the International Laboratory on Animal Diseases, Nairobi, Kenya, 9–13 February 1981. Current Topics in Veterinary Medicine and Animal Science Volume 14. The Hague, the Netherlands, Martinus Nijhoff, pp. 227–237.
- Radley D.E., Brown C.G.D., Burridge M.J., Cunningham M.P., Kirimi I.M., Purnell R.E. and Young A.S. 1975a. East Coast fever: 1. Chemoprophylactic immunization of cattle against *Theileria parva* (Muguga) and five theilerial strains. *Veterinary Parasitology* 1:35–41.
- Radley D.E., Brown C.G.D., Cunningham M.P., Kimber C.D., Musisi F.L., Payne R.C., Purnell R.E., Stagg S.M. and Young A.S. 1975b. East Coast fever: 3. Chemoprophylactic immunization of cattle using oxytetracycline and a combination of theilerial strains. *Veterinary Parasitology* 1:51–60.

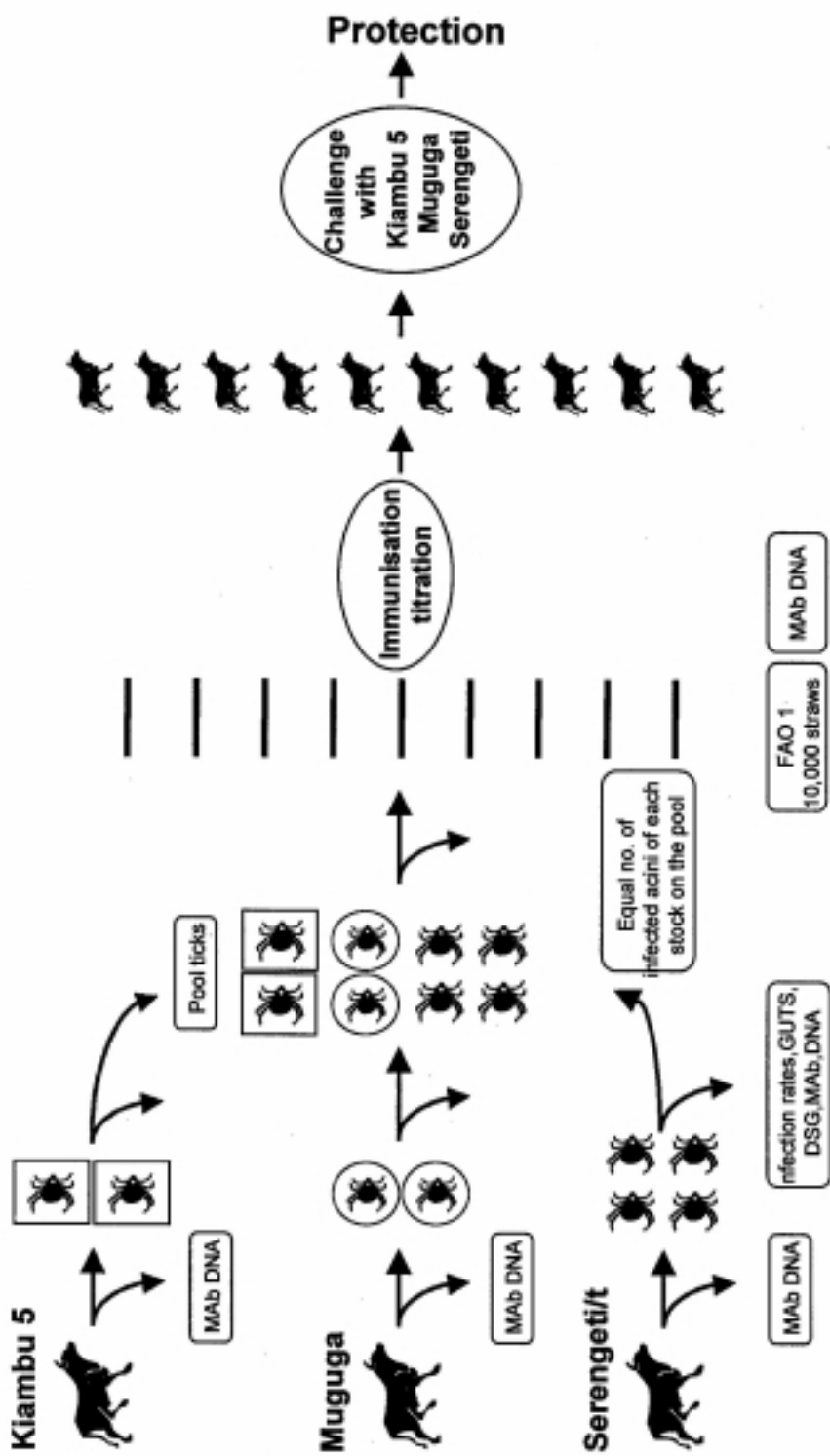


Figure 1. Preparation and evaluation of FAO 1.

Preparation of stabilates for immunisation against East Coast fever at NVRC, Muguga, Kenya

R. Payne

National Veterinary Research Centre (NVRC), Muguga,
P.O. Box 32, Kikuyu, Kenya

A marketing strategy to deliver East Coast fever immunisation using the Marikébuni stock (ECFiM) on an economically sustainable basis is being developed by the Kenya Agricultural Research Institute (KARI) and arrangements are under way to involve a private sector partner in this undertaking. The approach is detailed in a document prepared by the National Veterinary Research Centre (NVRC) entitled 'ECFiM-A Marketing Strategy'. This paper highlights some of the issues being addressed by KARI and the KARI/Overseas Development Administration of the United Kingdom (ODA) Tick-borne Diseases Project on the way towards commercialisation of stabilate production and immunisation.

Preliminary activities

Selection of an immunising parasite stock

The *Theileria parva* Marikébuni stock has been selected as the vaccine strain for use in Kenya. This decision was based on available information, which suggests a broad spectrum of cross-immunity against other parasite stocks.

Refurbishment of facilities and improvement of security at NVRC

In order to comply with commercially acceptable production standards, the facilities used for stabilate production will be refurbished. In addition, security fencing is being erected around each of the outside units; only designated personnel will be allowed to enter these areas and protective overalls of a colour specific for each unit will be worn.

Standards for stabilate production and quality control

Stabilates to be used for ECFiM will be prepared at NVRC, Muguga, in accordance with the standards for production described in the document 'Standards for Preparation of Stabilates for Immunisation against East Coast fever in Kenya'. A modified Standard Operating Procedure is being developed which takes these into consideration.

DVS approval

The standards for stabilate preparation have been approved by the Director of Veterinary Services (DVS), Kenya. The DVS will be kept informed of all relevant aspects of immunisation in the field.

Selection of a commercial partner

Putative commercial partners have been contacted and the process of selection is underway.

Laboratory activities

Stabilate production

Stabilates for field immunisation are prepared at NVRC from a seed stock of *T. parva*, Marikébuni stabilate 3014, originally isolated from the Coast Province of Kenya. Safety and quality testing may be done elsewhere, if an appropriate agency is identified.

Cattle for stabilate production

Cattle are purchased from a farm where ECF has not been reported for more than two years. Before purchase, sera are tested for antibodies to tick-borne diseases (*Theileria parva*, *T. mutans*, *Babesia bigemina* and *Anaplasma marginale*) and enzootic bovine leucosis (EBL) and blood smears are examined for haemoparasites. Only animals whose tests are negative will be considered suitable. Cattle are quarantined at NVRC in tick-free accommodation for two months. Acaracides are not applied to ensure low residual levels when animals are washed before tick application. Blood smears are taken three times a week during quarantine and examined for haemoparasites while serology to detect antibodies to *Theileria* is performed at four-week intervals. Steers of 250–300 kg body weight are used for tick-feeding, as they are more likely to survive the infection than smaller calves.

The severity of disease is a common constraint when feeding ticks on infected cattle, treatment with a curative drug or euthanasia may be required before a sufficiently high number of ticks have dropped. Treatment with a diuretic (fuzemide) has been found to relieve pulmonary oedema, one of the major symptoms, thus allowing tick-feeding to continue until completion. Cattle are treated with the antitheilerial buparvaquone once tick-feeding has finished.

Database

A database is being set up to accommodate clinical observations from cattle infected with the seed stabilate 3014, data on tick infection rates and stabilate infectivity. It is anticipated that this information will help to identify factors influencing tick infection rates and ultimately to produce tick batches of predictable and uniform infectivity.

Characterisation

In vitro: To check homogeneity of stabilates prepared from the seed stock, schizont cultures for characterisation studies are established routinely from cattle infected for tick-feeding and subsequently from the animals used to check stabilate viability after it has been frozen in liquid nitrogen.

In vivo: A panel of stabilates is being prepared from a number of *T. parva* isolates, these will provide infective material for standardised cross-immunity studies and will enable comparison of future batches of vaccine stabilates.

Field activities

Efficacy trials

Field trials are currently being conducted in different areas of Kenya to test the efficacy of ECFiM against natural challenge.

Monitoring field vaccination

All aspects of field vaccination will be monitored in conjunction with the commercial partner and field veterinarians.

Data on ticks and other tick-borne diseases (TBDs)

It is anticipated that tick control will be reduced on farms where cattle have been immunised. The effect of changes in dipping regimes on tick infestations and other tick-borne diseases (TBDs) will be assessed and the socio-economic benefits of such programmes analysed. Data on ticks and other TBDs will be collated at NVRC. This information will be made available to the DVS and used as a basis for the formulation of policies governing control of ticks and TBDs.

Responsibilities of NVRC and the commercial partner

ECFiM delivery

An appropriate and sustainable method of ECFiM delivery will be developed.

Training field veterinarians

Since October 1994, training of private veterinarians has been the responsibility of the KARI/ODA TBD Project. In the future, the commercial partner will play a role in recruiting and training veterinarians.

Extension to farmers

An extension package to help farmers appreciate the potential benefits and risks of immunisation is being designed and tested.

Technical support to veterinarians

Both NVRC and the commercial partner will have an ongoing responsibility to provide technical support to field veterinarians involved in the immunisation programme.

Advice on alternative tick control strategies

The possibility that tick control may be reduced in groups of immunised cattle is mentioned above. It is the responsibility of NVRC and the commercial partner to help formulate integrated tick and TBD control strategies.

Feedback of information to the DVS

All activities under this programme will be reported to the DVS. This includes the number of stabilates produced, areas where efficacy trials are being conducted, veterinarians trained, results from the vaccination programme, information on the incidence of ECF and results from investigations on ticks and other TBDs.

Application of molecular tools in support of deployment of *Theileria parva* live vaccines

R. Bishop,¹ V. Nene,¹ P. Spooner,¹ S. Mbogo,² D. Kariuki,² R. Payne² and S. Morzaria¹

¹ International Livestock Research Institute (ILRI), P.O. Box 30709, Nairobi, Kenya

² National Veterinary Research Centre (NVRC), Muguga, P.O. Box 32, Kikuyu, Kenya

Goals of characterisation

The primary goals of applying molecular tools, developed at the International Livestock Research Institute (ILRI), in conjunction with the use of infection and treatment immunisation are:

- quality control of vaccines
- monitoring of possible vaccine breakthrough and breakdown
- assessment of the impact of introducing new parasite types on long-term efficacy of the vaccine.

There are two main categories of tools available for use; serological, such as schizont-specific monoclonal antibodies which are now in routine use in some regional laboratories, and DNA-based, which are mainly used at ILRI but are likely to be deployed for routine use in regional laboratories in the near future.

Serological techniques

A panel of monoclonal antibodies (MAbs) has been raised against the schizont stage of the parasite. These are used in an immunofluorescence assay to determine which monoclonals react with the schizonts in antigen prepared from isolates in tissue culture. The reaction is scored as positive (+) or negative (-). The monoclonal antibody profile obtained is useful but does not discriminate all isolates. Most of the MAbs are against epitopes on the polymorphic immunodominant molecule (PIM) and therefore give limited information.

DNA-based techniques

Southern blotting

This technique generates unique fingerprints from multiple loci throughout the genome using cloned *Theileria parva* DNA sequences as probes. The fingerprints produced allow the discrimination of most *T. parva* isolates and, collectively, are useful for vaccine

characterisation. The method will only work on DNA purified from tissue culture of an isolate. Four *T. parva* polymorphic probes are currently available:

Tpr repetitive	Protein-encoding; expressed only in piroplasm stage
Telomeric	located at ends of chromosomes; detects variation at 8 loci
LA6 repetitive	Protein-encoding; expressed in schizonts and piroplasms
Minisatellite	Non-coding; at multiple loci on all chromosomes

Polymerase chain reaction (PCR)

In the polymerase chain reaction (PCR) method, specific DNA sequences are amplified and large quantities of target DNA may be produced and detected making this method important for diagnosis particularly where the quantities of *T. parva* in the samples may be minute, for example, in the blood of *T. parva* carriers. The method works on whole blood or tick salivary glands.

Single strand conformation polymorphism (SSCP)

Here, PCR is used to amplify DNA from different sites in the genome, followed by single strand conformation polymorphism (SSCP) to detect small DNA sequence differences (small genetic differences) at each of the sites (loci). This technique works on whole blood and is useful as a research tool for the direct analysis of *T. parva* populations in the field. Oligonucleotide primers are available to study 10 loci. Once amplified by PCR, the gene sequence at a locus can be characterised by SSCP, restriction enzyme digestion or direct nucleotide sequencing. The gene sequences involved are located on all four chromosomes and include antigen genes, such as PIM.

Quality control and markers for vaccine stocks

Using a combination of the above techniques, the vaccines in use can be identified and differentiated. Molecular characterisation has revealed that vaccine stocks, like field isolates, are heterogeneous. Application of this characterisation during vaccine production is an essential part of quality control.

It would be very useful if there were a unique marker for each of the vaccine stocks used which is not found in field parasites. Apart from being able to determine if an animal had been immunised, such markers would enable some important questions about the immunising parasite to be investigated, for example, whether it:

- replaces local strains resulting in a homogeneous parasite population
- undergoes sexual recombination with local strains potentially creating parasites with altered virulence or antigenicity

- alters significantly in genetic composition during tick/cattle passage for stabilate production.

Currently, vaccine markers are only available for Kiambu 5 and Marikebuni. They were derived from the 3 conserved region of Tpr. To apply them, PCR is used to amplify the sample and the PCR product, if present, is detected by hybridisation with the radio-labelled specific oligonucleotide.

Ongoing collaboration in a three-year project, funded by the Department for International Development (DFID) of the United Kingdom, between ILRI and the National Veterinary Research Centre (NVRC), Kenya, is addressing the issues raised above. The Marikebuni vaccine is being used in the infection and treatment method in Kenya and this research will continue alongside its use in the field. A large number of isolates will be characterised with MAbs and the full panel of DNA probes pre- and post-vaccination. A PCR-based marker is to be generated for the Marikebuni stock and will be used to test field ticks and unimmunised cattle for the presence of the Marikebuni stock. This project will also have a role in strengthening the capacity of NVRC staff to support immunisation programmes, through training.

Investigating possible vaccine breakdown and breakthrough

In situations where vaccine breakdown (failure to immunise) or breakthrough (immunisation successful but failure to protect against a field strain) are suspected, characterisation could assist the investigation, together with all the other relevant information about the disease outbreak. The PCR assay can be used on whole blood to establish whether the carrier state has been induced by the vaccine stock. Southern blotting can be used to examine the DNA of tissue culture isolates prepared from the same animals to check whether the animals have been infected with parasites other than the vaccine strain. At present, there is no *in vitro* method of characterisation that can predict whether cross-immunity will occur between the vaccine and another parasite. Therefore, characterisation is primarily useful in distinguishing breakdown from breakthrough.

Molecular epidemiology of ECF

The application of vaccine markers to studying the impact of the immunising parasite on the epidemiology of ECF has been mentioned above. Other areas in which molecular characterisation is used to study the epidemiology of theileriosis include:

Role of other *Theileria* species

PCR-based assays derived from ribosomal RNA or antigen genes are available to distinguish the various *Theileria* species that may be encountered in the field.

Genetic variation between *T. parva* isolates

The DNA from different isolates can be examined to determine whether the isolates are made up of several types or a single clone, and to study to what degree they vary from each other. Two examples of such studies are given below.

In Zimbabwe, in association with immunisation using the local Boleni stock, 40 isolates from 12 farms in buffalo-free areas were characterised using two probes. Most isolates appeared very similar using the Tpr probe. However, these could be discriminated from each other by the telomeric probe, which highlights the need for using a comprehensive panel of probes.

ILRI and ITM, Antwerp, collaborated to characterise Eastern and Southern Zambian isolates using all four DNA probes mentioned above. These revealed limited diversity, perhaps consistent with recent divergence from a single clone. A single isolate was obtained from Southern Zambia after deployment of Muguga cocktail vaccination. This appeared similar to Muguga with one probe and one SSCP marker but different with others. The significance of this is uncertain since the probes and markers have not yet been validated on a wide enough range of *T. parva* isolates.

Population genetics

These studies aim to quantify some parameters in the *Theileria* life cycle, such as, the frequency of multiple infections with different *T. parva* isolates in ticks and cattle and the extent of sexual recombination in field populations. The approach being used is to generate specific markers which differentiate field isolates and, also, to look at the frequencies of different polymorphic loci (using PCR-SSCP) and test whether they associate randomly. One significant outcome of this work will be that it will provide an insight into how rapidly resistance to a multivalent vaccine can evolve.

Standards for live tick-borne disease vaccines

T. Dolan

Livestock Services, P.O. Box 24720, Nairobi, Kenya

Standards have been published for live tick-borne disease (TBD) vaccines by the Standards Commission of the Office International des Epizooties (OIE) (OIE, 1990, 1991) and a revision is in press (OIE, 1997). In the preparation of these standards, specialists prepared drafts for each vaccine, which were then circulated for comments to the 114 member countries. Experts in these countries reviewed the drafts and, where appropriate, modified them before approval. The countries in the eastern, central and southern African region, together with the Food and Agriculture Organization of the United Nations (FAO), the Organization of African Unity/Inter-African Bureau of Animal Resources (OAU/IBAR) and the International Laboratory for Research on Animal Diseases (ILRAD, now the International Livestock Research Institute (ILRI)) also established a Standards Committee (in 1991) to prepare draft standards for these vaccines; particularly stabilate vaccines for East Coast fever (ECF).

Live vaccines for anaplasmosis and babesiosis are produced in Australia, South Africa and elsewhere, and for cowdriosis in South Africa, on a commercial or cost-recovery basis. Standards and an industrial attitude to quality assurance and safety have been in force for these products for some time. The stabilates used in infection and treatment immunisation against ECF have been more experimental in nature and generally produced in small batches. These stabilates are prepared using cattle and ticks, and are potential sources of extraneous infections. In addition, the concentration of infective material, *Theileria parva* sporozoites, will vary between batches and, when more than one stock is used in a vaccine, the implications of the relationship of dose of each component stock has to be addressed.

The use of infection and treatment immunisation is being vigorously promoted in the region as one element of integrated tick and TBD control. If ECF stabilate vaccines are to be used widely, they must conform to high standards of safety and reliability. Donor support for delivery of integrated tick and TBD control is a short-term option and private delivery must replace it, if it is to be sustained. The production of the vaccines must also be commercialised or at least be capable of cost-recovery. Thus, the whole process from production to delivery must become commercial in outlook. Products that are manufactured according to strict protocols and that meet defined standards of quality and safety are essential to achieve this.

A final draft Standards, drawn up by the Regional OAU/FAO/ILRAD Committee, was circulated to all countries and agencies involved in August 1996. Minor alterations were proposed and these were referred to the Task Force now in place to guide the restructuring of the new regional tick and TBD control programme. The standards for anaplasmosis, babesiosis and cowdriosis are based upon, and closely resemble, those already in place in

Australia and South Africa. The ECF standards are the result of years of accumulated experience and expertise in a number of national laboratories, ILRAD (ILRI) and the Vaccine Production Centre (VPC) in Lilongwe, Malawi, with input from consultants from protozoal and virological vaccine laboratories, supported through FAO.

The draft standards include recommendations on the sources and screening of cattle and rabbits, sources of ticks, seed stabilates of immunising stocks, the *in vitro* and *in vivo* characterisation of stabilates, and titration and safety testing of stabilates for use in the field. The list of organisms given in the standards that might contaminate stabilates is long, but not exhaustive. Each government must consider the screening it requires for disease security and for the safety of its cattle. It must also consider the national requirements with regard to characterisation of immunising stocks. The fact that some countries produce their own stabilates while others use stabilates produced at the VPC means that different standards are likely to be applied. The *in vivo* cross-immunity characterisation of a stock stabilate(s) may differ if the stabilate(s) is to be used in different countries or even different areas of a country, because of antigenic differences of parasites in the different areas. The decisions on safety screening and characterisation are direct responsibilities of the user governments and must be part of the contract with the production facility.

Infection and treatment immunisation uses potentially lethal stabilate(s) of *T. parva* and an oxytetracycline to control the infection. Countries in the region have different regulations regarding registration of veterinary products. The stock used for vaccination in Zimbabwe, *T. parva* Boleni, has been registered for use with and without tetracycline (under the name Bolvac). In other countries, the vaccine stocks have been approved by the respective Veterinary Departments but not formally registered. This may become an issue as countries develop, strengthen or enforce registration of such products.

In the production of the vaccine it is usual to test the efficacy of one long-acting oxytetracycline for the control of a particular stabilate batch. Not all long-acting oxytetracycline formulations perform equally even though the concentration of antibiotic in different formulations is the same. The vehicle may be different and therefore, the pharmacokinetics and pharmacodynamics may differ. One product is used routinely in producing each batch of the trivalent combination at the VPC in Lilongwe while two products have been shown to be efficacious in controlling the *T. parva* Marikebuni stabilate stock in Kenya. A product profile for the tetracycline was presented at the regional meeting held in Lilongwe in April 1994 (Musisi and Dolan 1997) and although the meeting agreed that commissioning a reasonably priced and specially labelled product for the region was a good idea, it did not make a specific recommendation as there was concern about limiting the provision of tetracycline to one company. Failure of a tetracycline to control the immunising infection has been recorded on more than one occasion and it is difficult to accept a vaccine package that does not contain a specific product(s) recommendation. If this is not possible, the package must contain a caution that the use of another product might lead to clinical reactions if it has not been shown to be effective before use in the field. The efficacy testing would be the responsibility of the user country or company, if the vaccine was being distributed and delivered privately.

Standards for live TBD vaccines are available and those published by the Standards Commission of the OIE. Once the Standards drawn up by the Regional Committee are

approved, they will be forwarded through FAO to the OIE and will be integrated into the OIE Standards (revised version 1996) in the next edition of the OIE Manual. It is then the responsibility of the Veterinary Department of each country to communicate its requirements to the production facility. The Standards may be modified over time to accommodate specific needs and to adopt new recommendations. For instance, the *in vitro* characterisation methods now in use for *T. parva* (see Bishop, this proceedings) may be replaced by newer and more specific methods as this is a rapidly developing field of research.

The only standard that is still being developed is on titration of *T. parva* (see draft Regional Standards, item 21). A three-stage titration has been proposed and is being evaluated with a new bulk combination stabilate produced for the Regional Project (VPC) by ILRI (see Morzaria and Spooner this proceedings). It may also be necessary to institute re-testing for stabilates stored for extended periods (perhaps five years) and a titration procedure used to determine if there has been decay. Also, there is a need for an independent review and inspection process of production facilities, their records and products. If industry becomes involved in production, rigorous enforcement of quality assurance and safety is expected to be in place, but independent inspection may still be necessary to ensure that standards of quality and safety are maintained.

References

- Musisi F.L. and Dolan T.T. 1999. (eds). *Tick and Tick-borne Disease Control in Eastern, Central and Southern Africa. Proceedings of a Joint OAU, FAO and ILRAD Workshop held in Lilongwe, Malawi, 25–28 April 1994*. ILRI (International Livestock Research Institute), Nairobi, Kenya. (In press.)
- OAU/FAO/ILRI Regional Committee. 1996. Standards for live tick-borne disease vaccines. OAU/FAO/ILRI (Organization of African Unity/Food and Agriculture Organization of the United Nations/International Livestock Research Institute. Nairobi, Kenya. Draft.
- OIE (Office International des Epizooties). 1990. *Manual of Recommended Diagnostic Techniques and Requirements for Biological Products*. Vol. II. OIE, Paris, France. (Chapter B5).
- OIE (Office International des Epizooties). 1991. *Manual of Recommended Diagnostic Techniques and Requirements for Biological Products*. Vol. III. OIE, Paris, France. (Chapters B10, B11 and B20).
- OIE (Office International des Epizooties). 1996. Theileriosis. In: *Manual of Standards for Diagnostic Tests and Vaccines*. OIE, Paris, France. pp. 321–330.

Summary of discussion: Vaccine production

Facilitator: C.G.D. Brown

Rapporteurs: S. Mbogo and M. Moran

The five papers presented in this session described many of the practical aspects of stabilate production and the associated activities required to maintain vaccine standards, such as characterisation of stocks and testing for contamination with extraneous organisms.

Method of stabilate production, storage and handling

At present, there is no standard protocol for stabilate production and it was agreed that it would be useful for the various groups making stabilates to meet and standardise their procedures. The participants emphasised the importance of storing seed stocks separate from other stabilates, and ideally also in other laboratories. These seed stocks should be well characterised *in vitro* and *in vivo*. It was suggested that characterisation of both seed and vaccine stabilates should be repeated every 5 years to determine the degree of decay during storage, how this might be done was not discussed. Issues proposed for further research included the influence of cattle breed on quality and quantity of the tick pick-up, and the need for an alternative method to replace manual plucking of ticks from rabbit ears. It was emphasised that the most susceptible breed of cattle and line of tick must be used and a separate tick line should be maintained in case the tick colony used for tick pick-up has a problem. Accurate standardised techniques for assessment of infection rates in ticks must be applied. Packaging of stabilate in straws for storage in liquid nitrogen was recommended. Many participants felt that the medium used for the grinding and the diluent is not user-friendly and need to be improved. In relation to this, it was considered that the viability of stabilate on melting ice should be determined and that there should be a system of monitoring pH changes in thawed stabilate and ensuring that the stabilate is discarded at the correct time.

Quality control and assurance

It was generally agreed that quality control monitoring requires internal and external expertise and independent assessors, that production and quality control should be separate, that independent OIE certified laboratories be used for certain tests and that the laboratory producing stabilate should provide a form of certification of the product with a guarantee of defined quality. Stabilate producing laboratories need experience of the use of stabilates in the field to enable backstopping. The importance of giving clear instructions on

how the product should be used to avoid inappropriate procedures being employed was also emphasised. The conditions recommended for maintaining stabilate after thawing e.g. on ice or at room temperature (Zambia) need to be clarified. Only one occurrence of an adventitious agent in an ECF vaccine was reported; this was an ILRI stabilate which caused abscesses. The problem of bovine leucosis virus contamination of blood vaccines in Australia and Zimbabwe was also mentioned. Anaphylactic shock due to tick antigens or the cryoprotectant has occurred following stabilate inoculation but incidence appears to vary, it was recommended that adrenaline should always be available to treat cases that arise.

Stocks, strains and characterisation

The main vaccine stocks in use are Marikebuni, Muguga cocktail and Boleni, each of these is characterised and contains a mixed parasite population. Numerous buffalo-derived *T. parva* stocks exist but are not well characterised. The methods of characterisation were reviewed in Bishop's paper and were again outlined prior to discussion; *in vitro* methods using monoclonal antibodies and DNA probes can be used to characterise parasites in vaccine stabilates, reactor animals, carrier animals and field cases; *in vivo* methods include infectivity testing (measures virulence), cross-immunity (indicates immunogenic types present) and infectivity to ticks. The primary reproductive rate of different stocks was mentioned as an area requiring further study. The need for a standardised approach to characterisation, particularly of vaccine stocks, was emphasised. It was considered important that vaccine stocks should be fully characterised, especially as the history of some immunising parasites is not entirely clear. The rationale behind selection of immunising parasites as potential vaccine stocks was discussed. Three important criteria are that the stock must have a broad spectrum of protection, must be blocked with recommended oxytetracyclines and must be easily produced, for example, good pick-up of infection in ticks. It was admitted that there is a degree of trial and error in identifying protective parasites since only a limited number of stocks can be tested in cross-immunity trials. In the case of Zimbabwe, it was thought that an important reason behind the selection of *T. parva* Boleni was its low virulence. The possible need to conduct a large cross-immunity trial using representative country stocks was raised. Another issue discussed was whether immunising stocks generate the carrier status in immunised animals. The Muguga component of the Muguga cocktail does not induce carrier status, while the capacity of the other two components, Kiambu 5 and Serengeti-transformed, to do so remains unknown. The other immunising stocks (Boleni, Katete, Marikebuni and Zanzibar) do cause carrier status to develop. The discussion then moved on to ask whether it would be better to work with a clone, containing a single parasite population, rather than a stock, which contains a mixed parasite population. Evidence suggests that, although a clone is likely to be easier to work with, it is unlikely to confer sufficient protection. Results from experiments using different parasite subpopulations (clones) showed that they were not protective, although not all the clones could be tested because of the expense involved.

Vaccine delivery

The infection and treatment method of immunisation: Practice and problems

S. Mbogo

National Veterinary Research Centre (NVRC)-Muguga,
P.O. Box 32, Kikuyu, Kenya

The infection and treatment method (ITM) is currently the only effective means available to immunise cattle against theileriosis, although laboratory results using recombinant antigens are promising (Musoke et al 1992). The method involves inoculation of live, potentially lethal parasites into healthy cattle followed immediately by an injection of a long-acting oxytetracycline (TLA) at 20 mg per kg body weight. A mild infection of *Theileria parva* occurs in the immunised cattle, which stimulates cell-mediated immunity to schizont-infected cells. This immunity can last up to 36 months in the absence of further challenge (Burridge et al 1972) but may be expected to become lifelong through boosting from the continuous challenge expected in areas where ITM is necessary.

Most of the problems associated with ITM (Morzaria 1996) and the possible solutions (Mbogo et al 1996) have been discussed in previous meetings. They include:

- The requirement for a cold chain: for the parasite to remain viable, it must be stored in liquid nitrogen and, unless the diluent is vacuum packed, it should be stored at -20°C which may not be readily available in the field.
- The fact that some animals suffer clinical theileriosis after ITM. This may be caused by using a low dose of TLA (poor estimation of weight), using expired TLA, using a higher dose of stabilate (incorrect dilution) or immunising immunocompromised or stressed cattle. In addition, if the vaccine is titrated in cattle, which are inherently less susceptible to theileriosis, it may cause disease when used to vaccinate more susceptible cattle. Therefore, highly susceptible cattle should be used for all production stages of the vaccine. If immunised animals were incubating the disease at the time of immunisation, clinical disease still results since immunity takes time to develop. In this situation, molecular and immunological tools differentiating the immunising parasite from field ones would be very useful. Approximately 3% of immunised cattle react after ITM. To identify and treat these animals, cattle are monitored during days 14–28 after immunisation. Prolonged monitoring after immunisation is useful as it helps to identify breakthroughs and allows studies on the effects of immunisation on the epidemiology of *Theileria parva* infections. However, for it to be effective, all relevant field data should be recorded.
- *Theileria parva* parasites establish a carrier state in immunised cattle. Since many different strains exist, immunisation can result in the introduction of new strains of parasites into an area. However, this may not be a major concern where cattle movement is poorly restricted. The introduced immunising parasite can undergo sexual

recombination with the resident parasite population and produce new genotypes that may not be protected against by the vaccine stock.

- By maintaining the vaccine live, contaminating pathogens may also be maintained. This may cause serious disease problems, particularly with blood vaccines.
- The presence of many immunological types which may not cross-protect. In addition, due to free cattle movement, these immunological types are not geographically distributed, indeed a single farm may have more than one immunological type of *T. parva*. Fortunately, about 70% homology occurs in cattle-derived parasites.
- The lack of *in vitro* cross-immunity tests to indicate potential for protection *in vivo* necessitates the use of cross-immunity trials. Since cattle are the only experimental animals that can be used, this becomes an excessively expensive exercise.
- The use of TLA may suppress some parasite subpopulations and thereby reduce the protective ability of the vaccine (Hove et al 1995). Unfortunately, there is no test to determine whether this occurs.
- There is inadequate transfer of maternal immunity by immune dams to calves, which necessitates immunisation of calves.
- ITM may occasionally fail to invoke protective immunity. This may be due to loss of vaccine infectivity as a result of exposure to heat, poor handling or excessive pH changes, all of which affect the viability of the immunising parasite. It may also be caused by the vaccine and challenge parasites having different immunological types or possibly by overwhelming challenge in times of stress.

Apart from the scientific problems discussed above, ITM has several other limitations. It requires more expertise than other immunisations for it to be safely delivered. Mistakes such as poor handling or dilution may result in either no protection or severe disease. Secondly, the facilities required for diagnosis of haemoparasites to identify reactors, breakthroughs and other TBDs and haemoparasites are not always available.

Farmers who plan to use ITM must be given information about the advantages and disadvantages of the method. They must understand that cattle are vaccinated against *T. parva* infections only and can possibly suffer, and even die, from other diseases. For field delivery of ITM as a private good, there are three major key players; namely, the farmer, the veterinarian and the veterinary department. The needs and expectations of these major key players should be met.

For farmers to request for ITM, they must be made to appreciate that the method is reliable, affordable, available, appropriate and sustainable (Irvin 1996). The delivery mechanism must also be efficient, convenient, socially acceptable and efficacious. Marketing of farm produce should be liberalised to attract better prices and enable farmers to cover their production costs, which could include purchasing ITM. Extension linkages should be improved and participatory rural appraisal meetings held to assist farmers in making decisions. Farmers should be helped to understand the root cause of their problems and the potential interventions available. For ITM to be sustainable, the farmers need to see clear benefits of the technology in terms of improved productivity, increased profits and reduced risk of ECF.

For veterinarians, however, delivery of ITM must be safe and effective to help improve their relationship with farmers and avoid litigation. They must be conversant with the technology related to ITM. The ITM must be robust and manageable and use equipment and drugs, which are readily available.

From the point of view of a veterinary department, ECF a notifiable disease is for the 'public good'. However, immunisation against ECF is for 'private good' with farmers meeting all associated costs. To allow farmers to get maximum benefits from ITM and therefore help create demand for it, the veterinary department should introduce a change of policy to allow adoption of alternative control strategies. The department should be prepared to change the current tick-control policy, which may mean abandoning the cattle-cleansing act.

In conclusion, in spite of the problems described above, ITM is currently the only effective method of immunising cattle against theileriosis. Indeed, it is advantageous to control theileriosis by an integrated method using a combination of tick control, chemotherapy and ITM, yet the ITM remains the cheapest component. The benefits of ITM include provision of effective long-term immunity, reduced reliance on acaricide use, and reduced risk of losing cattle to theileriosis.

References

- Burridge M.J., Morzaria S.P., Cunningham M.P. and Brown C.G.D. 1972. Duration of immunity to East Coast fever (*Theileria parva*) infection of cattle. *Parasitology* 64:511–515.
- Hove T., Musisi F.L., Kanhai G.K., Latif A., Masaka S., Munatswa F.C., Pegram R.G., Kamendo S.P., Quiroga J.C., Mwangondwe R. and Dolan T.T. 1995. Challenge of *Theileria parva* (Boleni) immunised cattle with selected East African *Theileria* stocks. *Tropical Animal Health and Production* 27:202–210.
- Irvin A.D. 1996. Future approaches to the control of ticks and tick-borne diseases in Africa and the role of the Food and Agriculture Organization/Multi-Donor Regional Programme. In: Irvin A.D., McDermott J.J. and Perry B.D. (eds), *Epidemiology of Ticks and Tick-borne Diseases in Eastern, Central and Southern Africa. Proceedings of a Workshop held in Harare, 12–13 March 1996*. ILRI (International Livestock Research Institute), Nairobi, Kenya. pp. 7–14.
- Mbogo S.K., Kariuki D.P., Rumberia R.M. and Sparagano O.A.E. 1996. Introduction of immunization against East Coast fever to the Kenyan farmer. *Proceedings of the WAVMI conference, Edinburgh, UK, July 3rd–5th*. pp. 221–226.
- Morzaria S.P. 1996. Monitoring the efficacy of immunisation against theileriosis caused by *Theileria parva*. In: Irvin A.D., McDermott J.J. and Perry B.D. (eds), *Epidemiology of Ticks and Tick-borne Diseases in Eastern, Central and Southern Africa. Proceedings of a Workshop held in Harare, 12–13 March 1996*. ILRI (International Livestock Research Institute), Nairobi, Kenya. pp. 103–114.
- Musoke A.J., Morzaria S.P., Nkonge C., Jones E. and Nene V. 1992. A recombinant sporozoite surface antigen of *Theileria parva* induces protection in cattle. *Proceedings of the National Academy of Sciences of the USA* 89:514–518.

Vaccine delivery in Uganda

R. Nsubuga-Mutaka

Livestock Research Institute (LIRI), National Agriculture Research Organisation (NARO), P.O. Box 24, Entebbe, Uganda

Following a series of successful trials during 1990–92, which showed that the trivalent East Coast fever (ECF) vaccine, Muguga cocktail, offered satisfactory protection against locally occurring strains of *Theileria parva*, Uganda decided to introduce the vaccine to private farms. Precautions were taken because the vaccine is live, contains parasite stocks foreign to Uganda and the target cattle for immunisation are valuable exotic and crossbred dairy populations. It was decided that the vaccination technology would be introduced in two phases; first by demonstrations in each district followed by delivery (with partial cost-recovery arrangement) and recommendations for adjustment in tick control.

Demonstration of ECF vaccination technology

A stepwise procedure has been adopted involving:

1. Training of selected staff from each district where the vaccine is to be used in all aspects of tick-borne disease control with particular emphasis on immunisation.
2. Sensitisation of interested farmers within the districts. The farmers are provided with all the basic information about the technology. The contribution required from those interested in participating is spelt out, the most important being the commitment to monitor the temperature of each immunised animal daily for 28 days.
3. Selection of farmers according to the following criteria:
 - farmers with at least 50% exotic blood in their cattle who practise dairy farming as a business
 - farmers personally involved in the day-to-day management of the farm
 - farmers with good management experience in carrying out regular disease control and record keeping
 - farmers with healthy, well-fed animals
 - farms with appropriate infrastructure, particularly crushes.
4. Demonstration of the vaccination technology is performed on selected farms belonging to the selected farmers, situated close together to ease monitoring. Between 40 and 160 cattle are immunised in each district that served to train staff and sensitise farmers. The demonstration includes:
 - standard preparation of the vaccine (thawing, equilibration for 40 minutes and mixing)

- measuring rectal temperature, while also training farmers to read a thermometer and record results
- estimating weight, calculating the oxytetracycline (OTC) dose and administering an intramuscular injection
- collecting blood for serum
- vaccine inoculating and applying ECF identification tags
- collecting data on animals (farm, animal number, breed, age, body weight, temperature, body condition, OTC dose given).

The details for each district are given in Table 1.

Table 1. *ECF immunisation demonstrations in Uganda.*

District	Number of demonstrations	Number of farms	Number of cattle immunised
Mukono	2	6	89
Mpigi	2	6	107
Mubende	2	5	141
Mbarara	2	5	173
Masaka	2	2	125
Jinja	1	5	31
Kabarole	1	8	155
Bushenyi	1	7	133
Tororo	1	4	47
Mbale	1	14	33
Kiboga	1	9	146
Hoima	1	8	152
Total	17	79	1332

5. Monitoring for 28 days:

- farmer takes and records daily temperature and monitors the animal's condition and reports any abnormal behaviour
- participating veterinary officer (VO) makes about 6 visits to ensure temperatures are being correctly taken and recorded (around day 4); to reassure farmers when the temperature starts to rise (around day 10); and, where necessary, to take blood/lymph node samples and treat animals (between days 14 and 22)
- project staff make 2–3 visits between days 14 and 35 to reassure and give more advice on expected reactions and to collect blood for serum on day 35 post-immunisation
- laboratory staff examines slides and carry out indirect fluorescent antibody tests on day 0 and 35 serum to determine serological status pre- and post-immunisation

- data analysis is carried out by project staff using data collected on immunised cattle and data recorded on temperature, treatment and laboratory charts. This assists in grading the animals' reactions to the vaccine: as non-reactors (NR), mild-reactors (MiR), moderate-reactors (MoR) or severe-reactors (SR).

The demonstration is usually closely followed-up on these farms to immunise any animals left out for any reason during the first round.

Delivery at partial cost recovery

Farmers who then wish to continue with immunisation initiated the second phase of the introduction of ECF vaccination. Particular interest was given to the safety of the exercise in this phase. The programme is organised such that farmers contribute US\$ 5 in advance for each animal to be vaccinated. This money covers the cost of the drugs used during immunisation and monitoring, transportation and subsistence for the VO. The project staff also assist with immunisation, blood collection and monitoring. However, the frequency of their visits can be reduced as veterinary staff and farmers gain experience. After three or four joint immunisation exercises, the field staff usually take over the task with minimal supervision. The immunisations delivered on a partial cost-recovery basis are indicated in Table 2.

Table 2. ECF immunisation at partial cost-recovery.

District	Number of programmes	Number of farms	Number of cattle immunised
Mubende	3	45	514
Mukono	3	16	158
Total	6	61	672

Tick control following immunisation

The project staff conducted an assessment of the requirements for post-immunisation tick control on four farms that were practising different forms of intensive tick-control pre-immunisation. These farmers agreed to reduce tick-control and were shown appropriate levels of tick infestation: project staff emphasised that tick engorgement should not be allowed (see Table 3).

From this exercise, it is recommended that a 50% to 60% reduction in tick-control would be safe. The extent to which the tick control can be reduced, mainly depends on maintaining adequate control against the appearance of the tick vectors for other tick-borne diseases. However, selection of appropriate tick control for immunised cattle must take into account the different farming systems, management practices, types of acaricides in use,

environment and tick populations present. In addition, success of ECF vaccine delivery also depends on the level of initiative shown by immunisation staff and the degree of confidence that the farmers have in this staff.

Table 3. Post ECF immunisation tick control assessment.

Farm name	Type of cattle (breed)	Grazing system	Tick control pre-ECF Immunisation (09/94)	Tick control assessment (post ECF immunisation)				
				26/05/95	23/08/95	04/08/95	25/08/95	06/08/95
Sebunya	50-75% exotic (Friesian)	Enclosed pasture, reduced tick numbers	Hand spray with Supona 1x/7 days (all year)	a) last tc b) tick nos.	a) last tc b) tick nos.	a) last tc b) tick nos.	a) last tc b) tick nos.	a) last tc b) tick nos.
				a) 6 days before Supona spray b) 1 R. App. Per animal. No eng. ticks, no A. var.	a) 13 days before Supona spray b) 2 R. App. Per animal. No eng. ticks few A. var.	a) 20 days before Supona spray b) 3 R. App. Per animal. No eng. ticks few A. var.	a) 20 days before Supona spray b) 3 R. App. Per animal. No eng. ticks few A. var.	a) 20 days before Supona spray b) 8 R. App. Per animal. No eng. ticks few A. var.
Sekitoleko	75-100% exotic (Friesian)	Enclosed pasture, reduced tick numbers	Dipping with Supona 1x/7 days (all year)	a) 10 days before Supona dipping b) 2.3 R. App. Per animal. No eng. ticks, no A. var.	a) 14 days before Supona dipping b) 4.1 R. App. Per animal. No eng. ticks, no A. var.	a) 20 days before Supona dipping b) 5.8 R. App. Per animal. min eng. ticks, few A. var.	a) 20 days before Supona dipping b) 5.2 R. App. Per animal. min eng. ticks, few A. var. and B. dec	a) 20 days before Supona dipping b) 10.6 R. App. Per animal. some eng. ticks, min A. var.
				a) 14 days before Bayticol b) 1 R. App. Per animal. No eng. ticks, no other ticks	Owner absent. No assessment.	a) 41 days before Supona spray b) 16 R. App. Per animal, some eng. ticks, few A. var.	a) 20 days before Bayticol b) 3 R. App. Per animal, no eng. ticks, other ticks	a) 16 days before Bayticol b) 1 R. App. Per animal. No eng. ticks, no other ticks
Musoke	50-75% exotic (Friesian, Guernsey, etc.)	Communal grazing, high tick challenge	Hand spray with Decatix 1x/7 days (all year) but too little solution/animal	a) 6 days prior Decatix spray b) 7.3 R. App. Per animal. No eng. ticks, several A. var.	a) 14 days prior Decatix spray b) 15 R. App. Per animal, many eng. ticks, several A. var.	a) 13 days prior Decatix spray b) 14 R. App. Per animal, many eng. ticks, several A. var., few B. dec	a) 6 days prior Decatix spray b) 3.8 R. App. Per animal, min eng. ticks, no A. var., few B. dec	a) 6 days prior Decatix spray b) 8 R. App. Per animal, min eng. ticks, min A. var., no B. dec

A. var. = *Amblyomma variegatum*
 B. dec. = *Boophilus decolatus*
 eng. = engorging

min. = Minimal
 R. App. = *Rhipicephalus appendiculatus*
 Tc = tick control

Vaccine delivery in Tanzania

M. Majaliwa,¹ J. Melewas¹ and G. Lynen²

1 Livestock Development, P.O. Box 9152, Dar es Salaam, Tanzania

2 Veterinary Investigation Centre, P.O. Box 1068, Arusha, Tanzania

Introduction

Veterinary services in many African countries are in a state of transition with an increasing call for the withdrawal of government from continuing to provide services and privatise the whole sector. In Tanzania, the transfer of certain activities, which in the past were traditionally carried out by the government, is being discussed among the various stakeholders; government, producers and private practitioners. This selective privatisation of veterinary services will involve establishing:

- a legal framework to streamline private and public (government) delivered veterinary services
- clearly defined obligations, responsibilities and roles for both public veterinary services and private practices
- regulatory mechanisms for the control and financing of a system where official health mandates are subcontracted to private veterinarians.

Delivery of East Coast fever (ECF) immunisation

Delivery of ECF immunisation is taking place in the Northern regions (Lakezone, Arusha, Kilimanjaro and Tanga), Dar es Salaam and Coastal regions, Morogoro region and the Southern highland regions of Iringa and Mbeya.

Immunisation is targeted at improved dairy cattle and their crosses on both commercial farms and in the smallholder sector. Farmers are reached through existing extension networks of dairy projects which have participated in tick-borne disease (TBD) workshops. The project is now at the stage where farmers request ECF immunisation, which is administered by selected and trained veterinarians and professionals.

At present, only government veterinarians and paraveterinarians are involved in delivering ECF immunisation services. The few private practitioners who have previously participated in the delivery have shifted interest from provision of clinical services to the more profitable ventures of consultancy and import of pharmaceuticals.

Two full-time veterinarians who receive transport support run the project. Eighteen veterinary professionals are also delivering ECF immunisation on a part-time basis and are responsible for organising the delivery themselves. Most of the veterinarians work individually, although some are organised into teams. Three paraveterinarians have likewise

been trained in the delivery of ECF immunisation in areas where the government veterinarians were few or were fully occupied with administrative functions.

The major constraints faced by the delivery veterinarians are:

- transport problems
- fear of immunisation reactors and time spent monitoring although this decreases with experience
- reluctance in some farming systems to pay for the services
- concerns about adopting a new technology.

Cost recovery

Cost recovery for ECF immunisation has made a promising start in Tanzania, with farmers paying between US\$ 9 and 17 for one immunisation. The delivery package, which includes vaccine, buparvaquone, oxytetracycline (OTC) and ear tags, is given to the veterinarians who pay 4000 Tanzanian Shilling (Tsh) (US\$ 7) per adult and Tsh 3000 (US\$ 5) per calf into the ECF account.

The veterinarians or the paraprofessionals associated with them collect revenue from smallholders and pay it into the ECF account. In the Southern highlands, the Heifer In Trust Project (HIT) helps pay for immunisation in the area. Smallholder farmers cover the price of the vaccine, while the HIT project pays for the delivery costs (transport and allowances). Most commercial farms pay directly to regional ECF accounts though there are marked delays in payment. Experience indicates that immunisation services are more readily paid for by the smallholder dairy sector, especially in those areas where ECF accounts for high mortality in the dairy population.

Sustainable cost recovery and privatisation

Sustaining cost-recovery mechanisms for livestock disease control, including ECF immunisation, in Tanzania depends on several factors:

- the existence of continued demand from the livestock sector for veterinary service
- the ability to meet the demand and provide the quality of service required
- the apparent cost of the service provided in relation to the economic value of the livestock industry
- the provision of parallel livestock development programmes aimed at increasing livestock farming returns through increased productivity, marketing etc.

The principle of cost recovery has been practised within the livestock industry in Tanzania, but with limited success. One of the main problems is the ability and/or willingness of some livestock owners to pay for the services. To date, most cost-recovery schemes have been introduced too rapidly and on too large a scale. Schemes that begin with gradual cost recovery help overcome initial resistance to payment, although, each price increase may still be faced with reluctance to pay.

The most important group of livestock owners is the pastoralists that produces most of beef and an increasing amount of the milk consumed in Tanzania. Pastoralists are, by definition, dependent on their livestock for survival and are thus prepared to invest in preventive and curative services to protect their 'living banks' and seek available services. The project has not started ECF immunisation in the traditional sector due to logistical problems although requests have already been received.

Second in importance to the pastoralists are the sedentary smallholder farmers with 2–3 zebu cattle. They practise mixed farming of livestock and crops. Until market conditions exist where livestock production is competitive with crop production in terms of 'farm-gate' economic return, this sector is unlikely to pay for a privatised veterinary service.

A relatively small portion of the livestock sector (less than 2%) is that of the smallholder dairy farms. Currently, this sector is the only group that is economically viable, and potentially therefore, with the pastoralists, likely to both want and be able to pay for a privatised veterinary service.

The fourth sector includes the Livestock Multiplication Units and commercial farms. Although not all of these are interested in ECF immunisation, they may in future provide the best opportunity for private veterinary practice due to the concentration of the animals. It will also be essential to involve livestock farmers, through their appropriate representatives and organisations, to solicit their views on the required type of animal health service.

Future objective: Improved cost recovery and increased privatisation

New models for the delivery of veterinary services are being developed for sub-Saharan Africa, involving an appropriate mix of state (public good sector) and private (private good sector) veterinary services. Privatisation of livestock services usually takes one of two forms; delivery by private veterinarians operating independently, or contracting of private veterinarians by the public sector. Contracting private veterinarians is often used to deliver services in production systems where the farmer-perceived value of the service is below the actual cost.

Personnel involved in the delivery of ECF immunisation

In Tanzania, with only a limited number of private veterinarians active in the delivery of clinical services, government veterinarians could be allowed to practise privately within the terms and conditions provided by the Tanzanian Veterinary Association (TVA).

Incorporating the traditional livestock sector in ECF immunisation could be achieved on a contractual basis, or by using government veterinarians based in duty stations along livestock marketing routes.

Whatever model is adopted, all envisage active participation of paraprofessionals in the ECF immunisation delivery. This must, however, be under the close supervision of the

veterinarian authorised to immunise. The use of trained and licensed paraprofessionals needs to be encouraged, in areas where no professional veterinarian is available.

The success of large-scale ECF delivery critically depends on proper support and remuneration of paraprofessional staff to ensure their continued motivation and effectiveness.

To provide support for the private delivery of ECF immunisation, instituting fully operational and effective Veterinary Investigation Centres (VIC) is also required.

Involvement of commercial agents in the distribution of the ECF immunisation package, extension of the cold chain, designing awareness campaigns and establishing close links with practitioners would be desirable.

Cost recovery and revenue collection

Evaluation of the current base-line delivery price will be carried out. Although veterinarians have been left free to charge their own farm-gate delivery prices, and this is a self-regulating exercise, a module on business management practices to new recruits needs to be incorporated into the training programme.

Existing revenue collection methods from ECF immunisation will be strengthened and a valid accountancy body needs to be established. Clear guidelines are required to ensure a transparent process.

Revenue collection and an expenditure control mechanism for the ECF fund may, in future, be controlled by the private sector.

Immunisation against theileriosis in Zimbabwe using the Bolvac vaccine without oxytetracycline

J. Mutugi

Food and Agriculture Organization of the United Nations,
P.O. Box 30750, Lilongwe 3, Malawi

An investigation was undertaken to validate earlier indications that at low concentrations *Theileria parva* Boleni sporozoites can be used for immunisation against January Disease in Zimbabwe without the usual concurrent oxytetracycline treatment. Groups of 50 susceptible Friesian steers from the same source were matched for weight and age and infected with varying doses of *T. parva* sporozoites (stabilate 24). This was to determine the most appropriate vaccine dose to use for immunisation without concurrent oxytetracycline blockage. After immunisation, cattle were challenged with potentially lethal inocula of homologous and/or heterologous *T. parva* sporozoites. The investigation, carried out during 3 immunisation experiments, spanned a period of 5 months.

Experiment 1

In the first experiment, 30 steers were randomly categorised into 5 groups (A–E) of 6 animals each and given 1.0 ml stabilate at dilutions of 1:10, 1:20, 1:30, 1:40 and undiluted, respectively. Group A animals were also injected concurrently with long-acting oxytetracycline (20 mg/kg as in standard vaccination method). The immunisation reaction was then monitored both clinically and parasitologically.

Cattle in groups A, B, C and D experienced no apparent immunisation reactions but developed significant antibody titres to *T. parva* in the indirect fluorescent antibody test (IFAT). Cattle in group E had moderate to severe theilerial reactions and required treatment. Group E cattle developed very strong serological responses to immunisation. The 30 steers were subsequently, challenged with a potentially lethal inoculum of the homologous stabilate together with susceptible controls.

All cattle in groups A, B, C and D responded to the homologous challenge with low-grade schizont parasitosis and transient febrile reactions. All underwent mild theilerial reactions and were considered immune. Group E cattle were all solidly immune to the challenge (no schizonts and no fever). Three months after the initial infection, the cattle were challenged with heterologous *T. parva* Avery, a highly virulent theilerial stock. All cattle were solidly immune to this potentially lethal heterologous challenge.

Experiment 2

In experiment 2, selected doses of *T. parva* Boleni, 1:10, 1:20 and 1:30 were investigated further to determine the most appropriate vaccine dose for use in the field. It was confirmed that all three dilutions could effect immunity to *T. parva* with no adverse theilerial reactions, and without the need for oxytetracycline treatment to obviate the vaccine reaction. Most vaccination reactions were not apparent although some cattle underwent moderate reactions in the lower 1:20 dilution of the vaccine.

Experiment 3

Experiment 3 was undertaken to select the optimal immunising dose from 1:20 and 1:30 dilutions. In the previous experiments, both dilutions gave a high degree of safety in cattle by inducing mainly no apparent theilerial reactions. However, following challenge of vaccinated cattle with the heterologous *T. parva* Avery stock, 93.4% and 75% of cattle inoculated with the 1:20 and the 1:30 doses, respectively, gave solid immunity to this potentially lethal challenge. The 1:20 dilution was deemed to provide better protection in cattle than the 1:30 dose, perhaps by inducing a better *T. parva* 'vaccine take' during the immunisation. Some of the reactions that were not apparent after immunisation with the 1:30 dilution, were later shown by heterologous challenge to have been in cattle, which were not, in fact, effectively immunised. This lack of uniform infection in cattle with the 1:30 dose is attributed to the higher sporozoite dilution and poorer vaccine take than with the 1:20 dose.

Following a successful validation trial, the 1:20 dilution of the *T. parva* Boleni vaccine (Bolvac) is now recommended for use in the field for *T. parva* immunisation, with no oxytetracycline chemoprophylaxis.

The Drug Control Council of Zimbabwe was satisfied with the results of the experiment and has registered this method of immunisation. Recently, some 13,000 cattle have been vaccinated this way in the field with no adverse reports. Since cross-protection has been observed by several workers between the vaccine stock *T. parva* Boleni and several representative virulent *T. parva* stocks from East Africa, it is strongly recommended that the Bolvac vaccine be explored for possible use in ECF immunisation.

The successful use of the Bolvac vaccine without oxytetracycline treatment for ECF vaccination, as reported in this study for January Disease, would constitute a quantum leap in minimising the adverse effects imposed by ECF on livestock production in the region.

Practical aspects of regional East Coast fever vaccine delivery

J. de Castro and M. Leneman

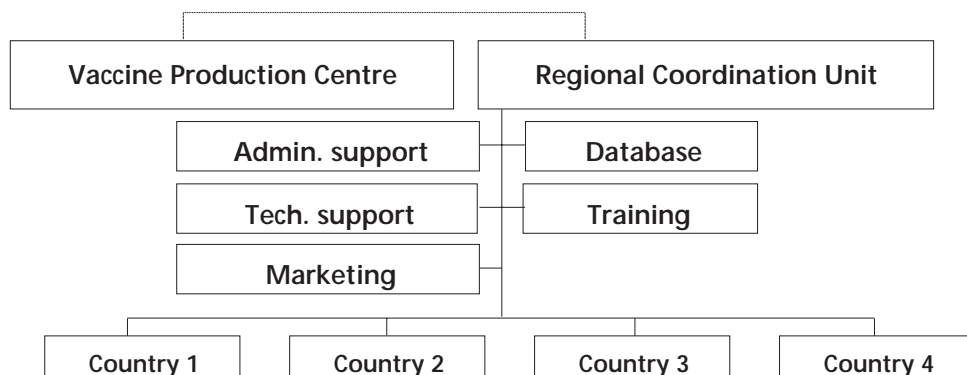
Food and Agriculture Organization of the United Nations (FAO),
Via delle terme di Caracalla, 00100 Rome, Italy

Background

For many years, the Food and Agriculture Organization of the United Nations (FAO) has advocated a regional approach to the control of tick and tick-borne diseases (TBD) in central, eastern and southern Africa with emphasis on the production and effective delivery of TBD vaccines, East Coast fever (ECF) vaccines in particular. The concept is based on the implementation of a regional programme for the integrated control of ticks and TBD. The programme, known as 'A Co-ordinated Multi-Donor Programme for Integrated Tick and Tick-borne Disease Control in Eastern, Central and Southern Africa', was developed in 1990. It was subsequently, endorsed in September 1991 by the FAO/Danish International Development Agency (DANIDA) Review Meeting, which recommended that: 'the proposed Regional Project (RAF/88/017, East Coast Fever Vaccine Production, Quality Control and Immunisation) should be re-designed as a pre-investment pilot vaccine production and delivery project'.

The Organization of African Unity (OAU)/FAO/International Laboratory for Research on Animal Diseases (ILRAD, now International Livestock Research Institute (ILRI)) meeting held at the same time also recommended that: 'a pre-investment (implementation) phase be initiated for TBD control by immunisation based on quality-controlled vaccines, produced on a commercial cost-recovery basis and delivered in a sustainable manner'.

A regional programme was then developed. Its structure is presented below.



The broad objectives of the programme were to establish an effective and a sustainable vaccine delivery system in the countries within an integrated national tick and TBD control programme, to train field veterinarians, laboratory staff and farmers in the delivery and monitoring of the use of vaccines and associated tick control methods and to develop a regional database on integrated tick and TBD control. The goal in the different countries was the creation of an effective, sustainable and environmentally friendly integrated tick and TBD control programme.

However, the donors that initially showed interest in the initiative (Belgium, Denmark and The Netherlands) disagreed with some of its components. To date, the programme has not been fully implemented, despite a dedicated vaccine production facility in Malawi, built and officially opened in 1994. At present, the programme is in a transitional stage developing a new structure and functions.

This paper emphasises the importance of maintaining a regional approach to TBD vaccine delivery. For a thorough analysis of vaccine delivery consult McDermott (1996); Musisi (this proceedings) details aspects of vaccine production.

Vaccine delivery: general considerations

The driving force for successful vaccine delivery is the existence of a demand for the vaccine. If the demand is there, cost recovery can be expected. An important aspect is that the governments are committed to the programme and help create a more enabling environment by adopting the immunisation methodology in their tick and TBD control policies.

The immunisation package to be delivered contains a live parasite and, in most cases, a long-acting tetracycline. Its delivery consists of a long and vulnerable chain of services and follow-ups. For the package to function successfully, it has to be as simple and robust as possible and with a high degree of flexibility. Most importantly, the delivery of the product has to be advantageous to all parties involved.

McDermott (1996) described different levels of vaccine delivery. These are the regional, national, provincial and farm levels. The comparative benefits determine who are the key players within the different levels. If the key players are made stakeholders of the process, it will greatly enhance the sustainability of the system, since their own success will then depend on the success of the delivery system as a whole. Linkages between the key players, especially with the farmers, further improve the functioning of the delivery chain (McDermott 1996).

Vaccine delivery: practical aspects

Even if the general conditions are met, there will still be several practical issues to be addressed before a vaccine delivery system has been established. The most important are mentioned below.

Vaccine delivery starts at the vaccine production site. A central production of ECF vaccines, apart from the obvious economic advantages, offers the best opportunity of having a concentration of technical knowledge and skills. This is beneficial, not only for the production and control aspects of the vaccine, but also to follow up the use of the vaccines in the field and to give assistance to the training of the users as part of its support services.

It leaves the various governments the chance to mainly deal with public goods such as the road infrastructure, support services, banking, and to set the import and export requirements. The exporting country of the ECF vaccine (i.e. Malawi) must meet the import requirements of the recipient countries. The product will then have to be registered under the relevant legislation in both the exporting and importing countries. The country exporting the vaccine must also be able to rely on a banking system that can receive and pay foreign currencies and carry out international transfers.

The governments of the vaccine-importing countries should provide a capacity for storing liquid nitrogen and an infrastructure, either existing or newly created, to distribute it. They too must have a reliable banking system and an immediate cash flow. Good communications between the exporting and importing country are, of course, of vital importance.

Regarding transportation, there is the need to obtain the necessary insurance, acceptable containers and to secure delivery and receipts of the consignments in time and under suitable conditions of conservation.

Finally, we should be aware that the delivery of any vaccine, be it for animal or human health, is a complex operation. In the case of the present ECF vaccine, the situation is further complicated by the need for liquid nitrogen, the vaccine's high cost and the possibility of post-immunisation reactions that require strict and careful monitoring. It is, therefore, crucial that the regional approach to ECF vaccine delivery is maintained to share regional facilities, experience and knowledge and, in this way, make vaccine delivery a viable option.

Reference

- McDermott J.J. 1996. Planning and evaluating delivery systems for the control of tick-borne diseases. In: Irvin A.D., McDermott J.J. and Perry B.D. (eds), *Epidemiology of Ticks and Tick-borne Diseases in Eastern, Central and Southern Africa. Proceedings of a Workshop held in Harare, 12–13 March 1996*. ILRI (International Livestock Research Institute), Nairobi, Kenya. pp. 125–132.

Commercialisation of vaccine delivery

N. McHardy

National Veterinary Research Institute (NVRI), Muguga,
P.O. Box 32, Kikuyu, Kenya

Summary

I gave a paper on the commercialisation of the 'Infection and Treatment System' of immunisation against ECF at a meeting in Malawi in 1994. It examined the likely attitudes of the pharmaceutical industry to the product and the demands they might make of its manufacturer, and it suggested the course that negotiations towards commercialisation might take. In Kenya, KARI has decided to pursue commercialisation of the product, ECFiM, and the process is conforming closely to those predictions. I presented the Malawi paper as a former Research, Development and Marketing Manager in a major pharmaceutical company, who had recently joined the ECFiM team at Muguga as a Technical Co-operation Officer (TCO). This time my paper is in the form of a memo that I might now write to my Commercial Director if I was still somewhere in the industry. I hope that it will raise some new points for discussion.

Subject: New product opportunity–Vaccine against East Coast fever

Background

About five years ago I ran an appraisal of an emerging technology to develop a vaccine against East Coast fever (ECF) of cattle in eastern Africa that the technology had demonstrated effective immunisation but it did not then constitute a marketable product. My opinion now is that a saleable product has been developed and I recommend that we should seek marketing rights to it.

The product

The product is a cryopreserved live suspension of the infective stage of the organism which causes ECF, harvested from the vector tick. It is injected into cattle together with long-acting tetracycline which 'blocks' the development of the infection. Vaccinated cattle develop a limited disease episode from which they recover and become immune to ECF for life. The system is unorthodox, but it is effective. Limited field trials have demonstrated acceptability among veterinarians and farmers. But:

- in about 5% of cattle the vaccine is not blocked by the tetracycline. These animals must be treated with a curative drug, but they do then become immune
- the vaccine does not confer 100% protection against ECF, (about 95% is claimed) and gives no protection against the closely related condition 'Corridor Disease' which is common in areas encroached by buffalo
- vaccinated cattle become carriers of ECF and are therefore a potential risk to unvaccinated cattle, though this is said not to be a significant problem under field conditions
- the product must be stored and transported in liquid nitrogen to preserve its viability. The veterinarian thaws and dilutes it, on farm, before injection while, we would be required to manufacture and supply the diluent
- field trials will not be completed until the end of 1997; while the development team is optimistic that these will be successful, there is a risk that the areas in which the product can be sold will be restricted
- the product has been developed in government laboratories, supported by aid funding. Manufacture is complex, requiring specialised facilities and expertise not available within the company. It is proposed, therefore, that manufacture should be contracted to the government laboratory. The company would have full access to the manufacturing process and product would be sold to us at cost plus a retained margin, perhaps 25%. Technical support to the product would be shared with the development team.

Analysis: With the exception of the possible restriction of market size, all of the difficulties are manageable. The opportunity is thus worth pursuing.

The market

ECF is a significant problem in eight African countries. The principal market would be in pure- and crossbred dairy cattle, which are very susceptible to ECF. About 60% of the market is in Kenya where a dairy cow is worth between US\$ 700 and US\$ 1000. At least 100,000 cattle die of ECF in Kenya each year. Veterinarians would deliver the vaccine. The dairying areas are fairly well covered by private practitioners, whose numbers are increasing.

Various appraisals of market size have been made, mostly by research workers, who tend to be optimistic. For Kenya alone, they suggest 100,000 doses in year 1, with a potential of rising to 500,000 doses in year 5. Commercial launch in Kenya should be achievable early in 1998, with test marketing in 1997.

Analysis: We should restrict our interest in the first instance to Kenya with a sales forecast of 20,000 doses in year 1, 100,000 in year 5 (low), and 50,000 in year 1, 250,000 in year 5 (high).

SWOT (Strengths, Weaknesses, Opportunities and Threats)

Strengths

Effective	95% protection is claimed
Single dose	Life-long protection
Safe	If used according to directions
Novel	First product in market sector
Acceptance	Product and price acceptable to veterinarians and farmers
Product fit	Fits existing range and capabilities

Weaknesses

Liquid nitrogen cold chain	Essential to maintain viability and efficacy
Safety	Dependent on strict veterinary compliance
Preparation	Need to prepare injection on farm
Spectrum	Not effective against Corridor Disease
Market size	Relatively small, possibly restricted
Withhold	Use of tetracycline requires 3-day milk withhold
Two-tier market	Veterinarian and farmer must be accessed

Opportunities

Increased market share	Large market sector of which we have a modest share
Customer contact	Increased tetracycline sales, and acaricides to small farmers
Leverage	Increased access to veterinarians and farmers
Image	Enhances image as an innovative company

Threats

Product conflict	Potential negative impact on acaricide sales to large farms
Product quality	Needs close monitoring of production in government facility
Veterinary input	Veterinarians will need technical and promotional support
Product liability	Responsibilities must be clarified
Bogus products	Product appearance, but not efficacy, easy to copy
Competing products	p67 subunit vaccine, at least 5 years from market—possibly over-hyped to attract funding

Analysis: ECFiM will need strong technical and marketing support. Monthly reports are essential. Agreement with Kenya Government must be watertight.

Positioning and promotion

ECFiM is a high-profile product. Veterinarian and farmer awareness needs to be streamlined to obviate some out-dated perceptions. Promotional literature for farmers and veterinarians, and a veterinarian training manual, have been produced and are already available. The name 'ECFiM' is established and should be incorporated in the company's product name. The existing pack is 20 doses but a smaller pack could be specified.

ECFiM will be sold to the veterinarian (primary market) who must also be helped to promote it to the farmer (the end user). Two positioning statements are, therefore, required. The author suggests the key word 'opportunity' for the veterinarian, to signify the opportunity to make additional money, based on these features and benefits:

Feature	Benefit
Radically new product	Opportunity to expand practice
Effective protection against ECF	Farmer will want to buy
Inexpensive compared with acaricide or drugs	Price acceptable to farmers
Supply from local depots	Product readily available
Easily applied	Opportunity for new practice recruits
Large potential market	Major profit opportunity

For farmers, the author suggests the key word 'security'. The features and benefits focus on the farmer's fear of ECF and the profitability of his/her business. If the farmer uses ECFiM, he/she will increase his/her security and income.

Feature	Benefit
Prevents ECF	Security against cattle dying, lower veterinary bills
Cost effective	More milk, more calves for sale, more money
Reduced tick control	Saves money, saves time
Protects exotic breeds of cattle	Security of investment in improved breeds

These promotional messages will be continually delivered through farmer and veterinarian meetings, at which our local company will provide the experts. The KARI team will be involved in promotion, and in veterinary training and technical support. The company should also produce a promotional video.

Analysis: ECFiM should be positioned as a novel element in an integrated strategy for the control of ECF, targeting both veterinarians and farmers.

Financial

ECFiM should initially be managed as a profit-neutral product. Financial benefit will come from increased sales of other products which are used in the 'ECFiM system', particularly tetracycline and through leverage to the rest of the company's range from increased access to farmers and veterinarians.

For the time being, the greatest uncertainty remains the effect that ECFiM will have on acaricide sales. The pessimistic view is that they will fall significantly, because a key element in the ECFiM promotion is reduced tick control using acaricide. However, ECFiM will make small farmers more aware of 'strategic' tick control that should make it possible at least to maintain acaricide sales in this sector. Acaricide sales will fall in the medium- and large-farm sector. However, penetration of ECFiM onto large farms will probably lag behind that on small farms because larger farmers will be reluctant to make radical changes to their strategies until they are convinced that ECFiM works.

The author's prediction, therefore, is that profit from products other than ECFiM in the smallholder sector will show a sustained rise in direct relation to sales of ECFiM. With the exception of acaricides, the same will happen in the large farm sector. Acaricide sales here will remain little changed while ECFiM sales are low, but they will fall significantly as ECFiM sales to the sector increase. Losses will then be off-set by increased profits from other products. Competitor's acaricide sales will, of course, show an uncompensated fall and could lead to an aggressive reaction. The financial appraisal is shown in the appended table.

Purchase price of ECFiM from KARI is likely to be US\$ 1.5–2.0 per dose. Manufacture and packaging of diluent will cost US\$ 1.0/20-dose vial, i.e. US\$ 0.05 per dose. Assumed cost of ECFiM plus diluent, therefore, is US\$ 2.0/dose. The author proposes a retained margin of 33% of selling price. Selling price to the veterinarian, therefore, is about US\$ 3.0/dose. Cost to the farmer essentially depends on the margin that the veterinarian takes to cover his inputs and professional fee. We must encourage the veterinarian to go for high volume sales rather than high profit margin.

Fixed costs total US\$ 419,000 to year 5. Pre-launch costs include a sales forecast, test marketing in Coast Province, establishment of a distribution system, diluent manufacture, staff training and product promotion. Promotion will be through veterinarian and farmer meetings, technical support and training for veterinarians, promotional literature for veterinarians and farmers, advertisements and features in the media, plus, of course, a launch seminar and party.

Analysis: ECFiM should be initially managed as profit-neutral over five years, assuming actual sales mid-way between high and low forecast. Financial benefit will come from increased peripheral business. Product failure would have an adverse effect on all other products, and company image. ECFiM, therefore, must be managed as a high risk product. However, the greater risk is that a competitor acquires ECFiM and makes a success of it!

ECFiM – Summary of costs and revenue (US\$ '000, 1997 constant)

Year (relative to launch)	-1	1	2	3	4	5	6 -10
Fixed costs							
Pre-launch work/test market	50	0	0	0	0	0	0
Distribution	40	30	15	20	20	20	100
Diluent manufacture	10	3	5	7	7	7	35
Technical support/training	20	30	15	15	15	15	75
Promotion/publicity	20	50	25	10	10	10	40
Total fixed costs	140	113	60	52	52	52	250
Low sales forecast							
Cost of product	0	(40)	(80)	(120)	(160)	(200)	(1000)
Fixed costs	(140)	(113)	(60)	(52)	(52)	(52)	(250)
Revenue	0	60	120	180	240	300	1,500
Balance (annual)	(140)	(93)	(20)	8	28	48	250
Balance (cumulative)	(140)	(233)	(253)	(245)	(217)	(169)	81
Interest on debt @ 20%	0	(28)	(47)	(51)	(49)	(43)	(34)
Balance, ECFiM A/c.	(140)	(261)	(300)	(296)	(266)	(212)	47
Profit, other products	0	10	20	30	40	50	250
Net balance (low sales)	(140)	(251)	(280)	(266)	(226)	(162)	297
High sales forecast							
Cost of product	0	(100)	(200)	(300)	(400)	(500)	(2500)
Fixed costs	(140)	(113)	(60)	(52)	(52)	(52)	(250)
Revenue	0	150	300	450	600	750	3750
Balance (annual)	(140)	(63)	40	92	148	198	1000
Balance (cumulative)	(140)	(203)	(163)	(71)	77	275	1275
Interest on debt @ 20%	0	(28)	(41)	(33)	(14)	0	0
Balance, ECFiM A/c.	(140)	(231)	(204)	(104)	63	275	1275
Profit, other products	0	25	50	50	50	50	250
Net balance (high sales)	(140)	(206)	(154)	(54)	113	325	1525

Financial summary

Scenario	NPV (US\$)	IRR (%)	Payback period (years)
Low sales forecast	140,000	2.7	8
High sales forecast	592,000	36.8	3.3

NPV = net present value; IRR = internal rate of return.

Summary of principal points

- The industry can handle ECFiM and they are aware of most of the difficulties.
- Distribution, support to veterinarians and promotion to farmers are manageable.
- The commercial partner will not necessarily seek to make a profit from ECFiM itself.
- Increased sales of other products and ‘market capture’ from competitors are key objectives.
- The biggest uncertainty in the industry is the effect of ECFiM on acaricide sales.
- The commercial partner will have a strong incentive to maximise ECFiM sales.
- Their sales forecasts for ECFiM will be conservative.
- ECFiM will alter the tick-borne disease (TBD) product market radically, whoever secures marketing rights to it.
- The potential effects of a competitor acquiring ECFiM are widely recognised, and feared.
- An integrated strategy and product range for ticks and TBDs is attractive to industry.
- If ECFiM fails, the whole business of its marketing company will suffer.
- Industry will demand a clear and robust agreement with the manufacturers of ECFiM.
- KARI’s negotiating position is fairly strong – the industry wants ECFiM.

But

- Some companies genuinely want ECFiM, others merely want to assess the threat it poses.
- The clever bit will be to distinguish between the two.

Because

- Selection of the ‘right’ commercial partner is critically important to the success of ECFiM.

Note: The views expressed in this paper are my own. It is unlikely that they are shared by either KARI or ODA. I hope, however, that a Marketing Manager in the pharmaceutical industry would be comfortable with most of what I have suggested.

Summary of discussion: Vaccine delivery

Facilitator: N. McHardy

Rapporteurs: L. Lynen and T. Dolan

The papers presented in this session highlighted the practical aspects of vaccine delivery in the field, the potential problems, the approaches taken to introduce the technology to new areas and the issues likely to be considered in commercialisation of ECF vaccine delivery.

Transport, thawing and diluting of vaccine

The issues of transport, thawing and diluting constitute important components of an efficient vaccine delivery system in the field. Participants pointed out that it is essential to avoid contact between water or moisture from outside the vial/container and the stabilate during thawing, dilution and dispensing. It was noted that when straws are used they could not be held in water/melting ice because they are open at one end. The Muguga cocktail stabilate, prepared at the International Livestock Research Institute (ILRI), is stored in straws and requires a different handling procedure for those accustomed to vaccine cryopreserved in tubes. Likewise, the need to improve the diluent was emphasised. The diluent should have good buffering capacity and contain a pH indicator. It should also be dispensed in capped bottles and not require to be stored frozen.

Selection of cattle for immunisation

When immunising calves of 1–2 months of age, care must be taken to assess their health and nutritional status. Calves with umbilical abscesses were reported to show a serious flare up of the abscess following immunisation. It is therefore important to provide clear advice to the farmers about management of calves so that immunisation is not compromised. Seropositive calves can, however, be immunised without problems.

On farms where strict tick control is maintained after immunisation, re-vaccination may be required after 36 months. Participants agreed that situations that allow immunisation of larger numbers of animals at the same time, e.g. multiplication centres, were the ideal places to apply immunisation. The panel also discussed potential complications during immunisation involving levamisole. Levamisole has been reported to ‘release’ the infection in situations where it was used during the week before or after immunisation using buparvaquone, thus causing a high reactor rate. There was, however, no recorded interaction when levamisole was used at the time as treatment during the development of buparvaquone. No such problem has been reported when using oxytetracycline for immunisation. More work may be required on this possible enhancing effect of levamisole and its potential influence on ECF treatment.

Vaccine inoculation and follow-up

Participants sought clarity which tetracycline should be used. They agreed that a particular oxytetracycline product should be recommended for use in the vaccine package. They also agreed that the efficacy of other products must be demonstrated before they can be introduced. The site of stabilate inoculation, either in the mid-neck or over the prescapular lymph node, was discussed. Some argued that since the nature of the tissues at the prescapular site varies between different breeds and types, they prefer the mid-neck area. Vaccination against other diseases should be avoided during ECF immunisation, while other TBD vaccines should not be applied during the 35 days following ECF immunisation. The impact of using more than one TBD vaccine in an integrated tick control was discussed at a Zimbabwe Veterinary Association meeting earlier and access to the outcome of the consultation would be helpful to all programmes in the region.

Delivery and price

Reduced delivery costs help sustain immunisation services. Such services are also economically sound when organised in the form of campaigns, routine vaccination rounds or immunisation to be performed in clusters of farms. Similarly, the use of promotional literature in helping farmers to take on ECF immunisation would certainly assist. Participants pointed out that the preparation of standard promotional literature was agreed at a meeting last year but had not been taken any further. Most countries have developed such literature, and it was felt that it would be helpful to exchange this information. Data recording systems should be standardised to provide useful feedback to departments of veterinary services (and commercial companies) to determine the uptake and impact of immunisation. This will also generate quantitative data for use in promoting and encouraging the use of immunisation.

Socio-economics and impact assessment

ECF immunisation in the smallholder sector: Points to consider in funding and sustainability

B. van Munster¹ and L. Lynen²

1 Tanga Smallholder Dairy Development Project, P.O. Box 1474, Tanga, Tanzania

2 Veterinary Investigation Centre, P.O. Box 1068, Arusha, Tanzania

Assumptions

A number of conditions are crucial for the success of immunisation against East Coast fever (ECF) in smallholder farms. Many of these issues have been addressed in other presentations. This paper concentrates on the final link of the delivery chain, from the regional store to the animal, which involves veterinary staff, paraprofessionals and village Animal Health Workers (AHW), farmers and their organisations. It is assumed that:

- the available product, the infection and treatment method (ITM) using the trivalent vaccine, will effectively immunise cattle against ECF if used according to the manufacturer's recommendations
- the delivery agent is able to answer basic questions from clients, for example, what tick control regime to follow after immunisation, how long the protective effect of the immunisation lasts under zero-grazing
- the delivery system guarantees the safe delivery of the vaccine up to regional level, where a storage facility is provided
- the store-gate price at regional level in Tanzanian shillings (Tsh) is 3000 (US\$ 5.0) for young stock and Tsh 4000 (US\$ 6.7) for adult cattle. The store-gate price includes the cost of the stabilate, oxytetracycline, buparvaquone treatment for an average 3.9% immunisation reactors, compensation for 0.03% deaths due to anaphylactic shock, import duties, transport, storage and cooling facilities
- the store-gate price is paid to the national delivery system, be it a private company, a non-governmental organisation (NGO) or a special revolving fund, which will ensure a continued supply of the items and facilities required for immunisation up to regional level.

Cost recovery

The system will only be sustainable if it is fully financed and if all stakeholders perceive that benefits (including financial ones) are derived from their involvement in the immunisation

activities. Otherwise, money leakage along the chain will sooner or later disrupt the system. The issue is not so much that full cost recovery is necessary to sustain the service, but more, who will pay the bill, and how to return the recovered money to the system. Farmers, government and donors (bilateral aid programmes, NGOs) should between them pay for the vaccine.

What is the cost?

The price for immunisation at the farm level will be determined by the following components:¹

- store-gate price at regional level
- payment of paraprofessional or AHW responsible for pre-immunisation extension visit and organisation of a sufficient number of animals to be immunised per visit (extension materials, transport, wages, allowances)
- payment of veterinary professional to carry out the immunisation (equipment, e.g. liquid nitrogen flask to carry stabilate, syringes, needles, slides, transport, remuneration)
- payment of paraprofessional or AHW responsible for post-immunisation monitoring (transport, wages, allowances)
- payment of veterinary professional to treat immunisation reactors (equipment, transport, remuneration).

How can the cost be reduced?

The price will be substantially lower if the actual immunisation and the treatment of reactors can be handled by paraprofessionals who, in many areas, are already responsible for diagnosis and treatment of ECF cases. This would probably help improve the sustainability of the system, especially in areas with no veterinary officer nearby. Because of the nature of the ITM, often perceived as complicated, and legal issues, this option has not yet been incorporated into the current policy.

Who will pay the cost?

Obviously, immunisation principally benefits the farmer, whose cattle run less risk of succumbing to ECF and who may save money otherwise spent on tick control and ECF treatments. Experience in Tanzania and elsewhere shows that urban farmers (who often have several cash earning activities) are willing and able to pay for immunisation, as long as they believe it reduces the ECF risk. Urban farmers pay up to Tsh 10,000 (US\$ 16.70) per immunisation.

1. The price for immunisation, equipment and transport.

A different situation exists in the rural and peri-urban areas, where the profit margins in a dairy enterprise are smaller because of a lower milk price and higher input prices. Even if the farmer believes in the profitability of the immunisation, shortage of cash makes it necessary to set other priorities. In addition, the cost of immunisation may be higher in these areas, as the veterinary professional will encounter higher transport and, possibly, accommodation costs.

The introduction of one standard price for immunisation would make the urban farmer subsidise his/her non-urban colleagues, which might not be appreciated by the urban farmers. Immunisation in non-urban areas could otherwise be subsidised (i.e. part of the cost donated) by aid programmes, NGOs or the government. This would help accommodate equity issues in the immunisation policy.

The farmers could pay either direct, or, where these exist, make their contributions through milk marketing organisations. These organisations would probably help facilitate collection of payments.

In Tanzania, payment for immunisation in two regions is through a Heifer In Trust (HIT) programme. The farmers pay a fee before they receive their heifer, which includes the regional store-gate price of the immunisation ingredients (Tsh 3000–4000). The programme covers the balance. HIT programmes could be encouraged to play such a role in immunisation activities in other areas as well. The farmer's contribution to the actual cost of immunisation could then be gradually increased.

Hidden payment of cost through quasi-privatisation

In Tanzania, immunisation is carried out by government employees. The few existing private veterinarians either have not engaged in immunisation activities or otherwise had started and then stopped. The official policy aims at shifting animal health services that are for the 'private good' (which would include ECF immunisation) to the private sector. Private professionals will only step into the vacuum created by a withdrawing government service if their activities are likely to be profitable.

In the meantime, government employees provide services in return for personal payment which increases their low income. Relying on government facilities and the privileges of their position also gives them an advantage over their private competitors. Government employees fulfil part of the demand for animal health services by making it more difficult for private veterinarians to reach the minimum level of business required to earn a reasonable income. Thus, this quasi-privatisation hinders the growth of strong private sector service providers and undermines the sustainability of the system.

Private practitioners could be stimulated to take part in the delivery of immunisation, if donors or government covered part of their costs. The subsidy could be an estimated equivalent of the amount the government puts into its employees' private activities (transport, equipment, salary).

Do benefits warrant the cost?

It is essential that all stakeholders perceive their involvement in the immunisation delivery system as beneficial. For professional staff the benefits will be primarily financial, although successful immunisations may increase farmers' confidence in the practitioner. Farmers will appreciate reduced ECF risk and reduced expenditure on tick control and treatment of ECF cases.

The success of ECF immunisation will essentially depend on a clearly demonstrated efficacy. This again requires an effective means of advertising. Long-term monitoring (exceeding the 40 days after immunisation) must therefore, be carried out either by the government, special task forces or the manufacturer, while financial assistance can be obtained from donor agencies.

If farmers see the benefits of immunisation, they will pay for the services. A follow-up of a small group of immunised animals in Tanzania, revealed that the ECF morbidity in immunised animals was twice as high as that in non-immunised animals on the same farms. One may argue that this is due to poor diagnosis. Immunised animals, become piroplasm carriers, and if they fall ill for any reason since laboratory diagnosis is often restricted to blood smears, they may, wrongly be diagnosed as suffering from ECF. Nevertheless, in most cases the animals had been treated with costly buparvaquone, at the expense of the farmer. Misdiagnosis or not, these farmers will no longer believe in the beneficial effects of immunisation.

Training professionals, whether they are involved in immunisation or not and increasing the knowledge of farmers, is therefore, vital to prevent the situation described above. Such training is likely be funded by the donor community.

Animal health delivery system

The ITM has often been linked to the privatisation of veterinary services. Indeed, the delivery of ECF immunisation to smallholder farms can only be consolidated if a cost-effective and sustainable delivery system for animal health services is established.

A key feature in planning the delivery system will be to co-ordinate tick-borne disease control with the provision of other services to livestock farmers (Walse et al 1991, cited in McDermott 1996). Immunisation activities may act as a catalyst in further developing a system for provision of services and disease monitoring.

References

- de Haan C. and Bekure S. 1991. *Animal Health Services in Sub-Saharan Africa. Initial Experiences with Alternative Approach*. World Bank Technical Paper 134. World Bank, Washington, DC, USA. 49 pp.
- McDermott J.J. 1996. Planning and evaluating delivery systems for the control of tick-borne diseases. In: Irvin A.D., McDermott J.J. and Perry B.D. (eds), *Epidemiology of Ticks and Tick-borne Diseases in Eastern Central and Southern Africa. Proceedings of a Workshop held in Harare, Zimbabwe*,

- 12–13 March 1996. ILRI (International Livestock Research Institute), Nairobi, Kenya. pp. 125–132.
- Mitchell M. 1996. Commercialisation of delivery systems. In: Irvin A.D., McDermott J.J., Perry B.D. (ed), *Epidemiology of Ticks and Tick-borne Diseases in Eastern Central and Southern Africa. Proceedings of a Workshop held in Harare, Zimbabwe, 12–13 March 1996*. ILRI (International Livestock Research Institute), Nairobi, Kenya. pp. 133–138.
- Odeyemi I.A.O. 1997. Understanding privatisation of animal health delivery systems in Africa: concepts, impacts and appropriate approaches. FAO (Food and Agriculture Organization of the United Nations). Electronic conference for rational delivery of public and private veterinary services (in press).

Estimating demand for theileriosis vaccines

A. Mukhebi¹ and T. Williams²

1 International Livestock Research Institute (ILRI), P.O. Box 30709, Nairobi, Kenya

2 International Livestock Research Institute (ILRI- Ibadan), c/o IITA, PMB 5320, Ibadan, Nigeria

Demand and supply for a vaccine

Demand refers to the amount of vaccine that buyers are willing and able to purchase at a particular moment at each possible price. Demand is more than a desire to purchase; it is the ability to purchase as well. Supply describes the amount of a vaccine that sellers (producers) are willing and able to sell at a particular moment at each possible price.

Demand and supply relationship

The relationship between demand and supply for a vaccine can be explained by Figure 1.

At a high price, P_1 , the buyers will demand a lower quantity, Q_1 , but the suppliers will be willing to supply a higher quantity, Q_2 . Similarly, at a lower price, P_2 , the suppliers will be willing to sell a lower quantity, Q_3 , but the buyers will demand a higher quantity, Q_4 . When the quantity supplied is equal to the quantity demanded, an equilibrium exists (Q_e) and the price that the buyers will be willing to pay will just equal the price at which the sellers will be

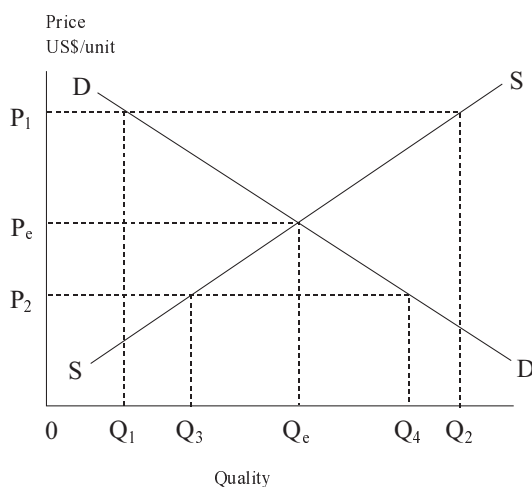


Figure 1. Relationship between supply and demand for a vaccine.

willing to sell (P_s). The price of a vaccine will, therefore, affect the amount that buyers are willing and able to buy, and the amount that sellers will be willing to offer for sale.

Other determinants of demand and supply for a vaccine

In addition to the price of the vaccine, there are other factors that will affect the demand for or the supply of a vaccine. These factors, which may shift the demand curve up (increase demand) or down (decrease demand), include:

- the price (cost) of a substitute (another vaccine or an alternative control option); the higher the price of the substitute, the higher the demand for the vaccine and vice versa
- quality of the vaccine (efficacy, safety, packaging); the higher the quality, the higher the demand for the vaccine,
- economic cost (impact) of the disease constraint; the higher it is, the higher the demand for the vaccine,
- production system; the more commercially oriented, the higher the demand for the vaccine,
- transaction costs; the higher the transaction costs (of access or delivery), the lower the demand for the vaccine,
- number of boosters per animal vaccinated; the greater the number, the higher the cost per vaccinated animal and the lower the demand.

The supply of a private good (whose benefits accrue to individuals rather than to the public) will depend very much upon demand, as well as on the cost of production, which may shift the supply curve up (decrease supply) or down (increase supply). For a public good (whose benefits accrue to the public rather than to individuals), supply (usually by government) will depend upon the social benefits to be gained rather than on demand. A vaccine for theileriosis is more of a private than a public good.

Estimating potential demand

Method

An attempt has been made to estimate the potential demand for the infection and treatment method (ITM) of immunisation against theileriosis. The method is based upon estimated numbers of cattle at risk from theileriosis in each of the 11 countries (determined by Geographic Information System (GIS) from the distribution of cattle and *Theileria parva* in each country), and assumed rates of adoption for the vaccine. In this analysis, national cattle populations were assumed to be homogeneous with respect to the effects of theileriosis. More detailed analysis of the potential demand for vaccine in selected countries (Kenya, Tanzania, Uganda and Zimbabwe) is currently under way at the International Livestock Research Institute (ILRI) in which an attempt is made to differentiate cattle populations by breed and management system. Using a computer-based spreadsheet model and the Food and Agriculture Organization of the United Nations

(FAO) Production Yearbook for national cattle populations for 1994, estimates of the potential demand for the ITM were obtained by country and for the whole affected region (Table 1).

Preliminary results

Estimates of the annual potential demand for an ITM vaccine by country and for the whole region are given in Table 1. A basic assumption was made that 80% of the calf population (less than one year) and 10% of the cattle population above one year are at the risk of theileriosis and would be targets for immunisation. Out of an estimated cattle population of nearly 65 million head in 1994, 38% are estimated to have been at risk of theileriosis, with 11% (about 7 million head) being the target number for immunisation by ITM.

Table 1. Country and regional estimates of the annual potential demand for a theileriosis vaccine.

Country	Cattle population 1994 ($\times 10^3$)	Population at risk		Target population for ITM*	
		No. ($\times 10^3$)	Total (%)	No. ($\times 10^3$)	Total (%)
Burundi	420	420	100	120	29
Kenya	12500	8263	66	2407	19
Malawi	980	738	75	210	21
Mozambique	1270	622	49	177	14
Rwanda	454	447	98	127	28
Sudan	21750	413	2	118	0.5
Tanzania	13376	4073	30	1160	9
Uganda	5150	4267	83	1011	20
Zaire	1475	255	17	73	5
Zambia	3300	2333	71	664	20
Zimbabwe	4300	3094	72	881	20
Total	64975	24925	38	6948	11

ITM = Infection and treatment method.

Table 2 provides the estimate of the annual potential demand for an ITM vaccine for the whole region assuming different rates of adoption. In the early years of vaccine availability, it can be assumed that the adoption rate would be low, but would steadily increase over time as livestock keepers become more aware of the availability of the vaccine and the benefits (if any) from its application.

Table 2. Regional estimates of the annual potential demand for a theileriosis vaccine at different rates of adoption.

Calves for ITM (%)	Cattle 1 year for ITM (%)	Total cattle for ITM (million head)
10	1	0.8
20	2	1.6
30	3	2.4
40	4	3.2
50	5	4.0
60	6	4.8
70	7	5.6
80	8	6.4
90	9	7.3
90	10	7.5

ITM = Infection and treatment method

Future research needs

The more detailed study mentioned above needs to be continued in order to cover a representative number of countries in the theileriosis region. The results would be used to help extrapolate the potential demand for the vaccine for the whole region. Country case studies of past ITM immunisation sites should be undertaken to establish effective demand and supply pattern for the vaccine. Such studies will also yield valuable data on the impact of the ITM at the farm level.

Assessing the impact of ECF vaccination: Implications of structural adjustment

A. McLeod

Kenya Agricultural Research Institute (KARI),
P.O. Box 30465, Nairobi, Kenya

Introduction

It is a daunting task to write about impact assessment given two detailed previous research papers on the subject by Perry (1996) and Mukhebi (1996) at the Tick-borne Diseases Meeting in Harare, Zimbabwe, and a number of relevant points dealt with by McDermott (1996), and during working group discussions.

Among the issues raised at the Harare meeting were: the apparent impact assessment; the importance of a client or stakeholder focus; the need for careful selection of impact indicators; the variation in impact with production system and disease risk; the need for and lack of relevant data; the need to prepare for impact assessment in planning and training. Looking at this comprehensive list, at first it seemed as if there would be nothing new to discuss. However, the author believes, it may be possible to add something to the ongoing debate by developing a theme around what McDermott (1996) referred to as the rapid changes in animal health services delivery. The issue was widely debated in the recent e-mail conference on 'principles for rational delivery of public and private veterinary services' organised by the Food and Agriculture Organization of the United Nations (FAO). Contributors to this conference have published papers on animal health services delivery; papers presented by policy makers at the Kenya Agricultural Research Institute (KARI) workshop on structural adjustment in 1996, and at the Intermediate Technology Development Group (ITDG) veterinarians' workshop discussed the progress of structural adjustment in Kenya.

In this paper I intend to briefly highlight restructuring in the animal health sector and examine some of the implications of the process on impact assessment of East Coast fever (ECF) vaccination. Apparent short-term measures have also been suggested to reflect the present transitional state. Hopefully the paper will stimulate lively discussion during working group sessions and encourage the formulation of relevant and topical proposals for action.

Effects of restructuring

The majority of sub-Saharan Africa is undergoing some form of structural adjustment that reflects on the form and function of animal health delivery. It would, however, be naive to

suggest that all veterinary services in sub-Saharan Africa are at the same stage of evolution or that they will follow the same development paths. But, it was apparent in the FAO e-mail conference that certain concerns are shared by many.

Structural adjustment, whether subscribed by the International Monetary Fund (IMF) or spontaneously adopted, generally involves downsizing and a shrinking budget for public services combined with an increasing demand for greater accountability and the requirement to encourage the growth of the private sector. The expected impact on the animal health sector involves downsizing the government veterinary service to a lean, mean advisory and regulatory body, with responsibility limited to public good elements of animal health care, some of which may be contracted to the private sector. Any elements which are clearly private goods will be taken over by a range of professionals and paraprofessionals in the private sector, functioning in a policy environment designed to create competition and accountability.

However, animal health care delivery services in sub-Saharan African countries are in a fluid state with neither the public nor the private sector functioning as the country would wish them to.

Implications for impact assessment

This section takes two themes related to structural adjustment and suggests the likely effects on impact assessment when restructuring is well-advanced. Some of these effects can already be observed in Africa while others can be predicted from common sense or experience in the developed world.

As the public sector budget diminishes, fewer resources are available to collect data, yet at the same time there is a demand for greater accuracy in assessment and prediction.

Implications

- Impact assessment will take a high profile. Strategic research into a vaccine is generally funded by public money and here financing will become increasingly dependent on the ability of the researcher to demonstrate high returns to investment. In countries that choose to deliver the vaccine through the public sector, the competition with other technologies will be fierce. If decision is made instead to distribute and deliver through the private sector, manufacturers and veterinarians will want to be convinced of safety, efficacy and adoption potential.
- The use of models will assume increasing importance. They must be used creatively for *ex ante* assessment and to highlight critical data—those to which the model is most sensitive. This has implications for training and recruitment since three specific types of expertise are required: a) modellers and model users, whom it may be more efficient to recruit than to train, b) data collectors, who need to understand the use and data requirements of a model but not the complexities of its design, and c) policy makers, who need to understand the output of economic and biological models but not their design or the data gathering process.

- The choice of indicators will be crucial as will the level of precision required in their estimation; where resources are constrained ‘need to know’ becomes much more important than ‘wish to know’. Choices need to be made between empirical and contingent evaluation methods, longitudinal and cross-sectional studies. For example, production data are needed for any economic assessment and longitudinal monitoring is undoubtedly the best collection method for accurate and detailed data. However, longitudinal monitoring is resource-hungry and, in most countries, can only be conducted in a few carefully chosen sites as part of a larger research effort.
- The choice of impact zones will be very important because this affects the efficiency of data gathering. Where the data collection system is resource-constrained, it makes sense to select high disease risk areas and target groups where considerable benefits can be expected, on the assumption that if these groups do not generate a sufficiently high rate of return it will be pointless to examine more marginal areas.
- Minimum data sets may be adopted for certain types of impact assessment. This is an elegant concept that has instant appeal to researchers and policy makers, but there are few successful examples. Taken in conjunction with the three previous points, a minimum data set would contain data from critical impact zones, on critical indicators, and would focus on variables needed to run models. However, the word ‘minimum’ tends to conceal the enormous effort needed to organise and sustain any database. It will be argued below that minimum data sets are most appropriate for public sector data and that it may be hard to introduce a standard format.

Government veterinary services are relinquishing their role as providers of clinical services and taking on a regulatory function. Correspondingly, the private sector is becoming increasingly important in distributing and delivering private goods. Arguably ECF vaccination, with its few externalities, can be made exclusive, is in high demand and is a private good (the case of immunisation and treatment is less clear-cut because of the carrier state). The direct involvement of the public sector in production and distribution of vaccines should be minimal in a liberalised market.

Implications

- Different stakeholders will have widely different and often competing needs for impact assessment (the government will place emphasis on safety–efficacy and environmental impact— and possibly on quality of life of consumers—social impact; private distributors will concentrate on information necessary for marketing; research financiers will continue to require the traditional assessments of returns to research). Since data collection will be done by stakeholders for their own monitoring and evaluation purposes, the quality and quantity of data collected can improve. The total cost of data collection under a free market economy is likely to be higher than that under a centralised economy but its quality is ensured.
- At the same time, the availability of data will decrease. Players in a competitive market may be willing to invest in data collection but unwilling to share it with others, for reasons of industrial secrecy and the understandable reluctance of the private sector to

appreciate the obligation to provide information to the public. Governments will need to be very clear on the required standard methods of data collection and analysis, while also demanding the return of information by the private sector to the government. Regulations concerning the accessibility of data, with the understanding that public control of or access to data has an associated management cost, need to be clarified.

- The need for and funding of impact assessment data may be separated from its collection. This is analogous to the delivery of the ECF vaccine, where each country will make its own decision about the balance between private and public financing, but in the absence of a large public field service, delivery must be made by the private sector. The same reasoning applies to impact assessment data. Even those elements required for government regulation may need to be contracted out to third parties: where appropriate these will be private animal health professionals, for certain data it will be necessary to employ neutral third parties.
- It will become more difficult to standardise data collection, storage and analysis for anything but public goods—regulation and quality control. In consequence, a minimum data set might concentrate on safety, efficacy and environmental impact and contain very little economic data. Private sector stakeholders might decide to standardise or share methods for reasons of efficiency but it will be difficult and ultimately unproductive to constrain them to do so.

It follows that a possible scenario could be where each government held a modest minimum data set to an internationally approved design, with the maximum use of models, a modest routine data collection effort and rapid turnover of information. Research would follow a system similar to the present funding approval process in the Consultative Group on International Agricultural Research (CGIAR) and international development community. At the same time, the private manufacturing and delivery sector would take charge of impact assessment for marketing purposes with sharing of certain data made mandatory and the accuracy of published results screened by third parties. Farmer groups would carry out their own impact assessment with the help of public or private extension agents. This could, however, be kept for the future.

The immediate need is for an impact assessment which addresses the limitations and fluctuations of existing animal health sectors. During this transition phase, it will be important for each of the stakeholders to recognise their own comparative advantage and future needs and concentrate on those elements of the system, while at the same time making efforts to collaborate and share information. To some extent this was anticipated from the conclusions of the Harare workshop. Our workshop could take the process a step further, by defining more clearly what is expected of each stakeholder and making a plan to achieve it. It can probably be assumed that impact assessment methods for research are well developed and that priority should be given to: the changing role of the government and the information that its minimum data set will be expected to supply; the training needs for those moving to the private sector to be able to take responsibility for their own impact assessment; and the impact assessment needed for the consumer during and after the transition.

References

- FAO (Food and Agriculture Organization of the United Nations). Electronic conference on 'Principles for rational delivery of veterinary services' January–March 1997.[In press].
- McDermott J.J. 1996. Planning and evaluating delivery systems for the control of tick-borne diseases. In: Irvin A.D., McDermott J.J. and Perry B.D. (eds), *Epidemiology of Ticks and Tick-borne Diseases in Eastern Central and Southern Africa. Proceedings of a Workshop held in Harare, Zimbabwe, 12–13 March 1996*. ILRI (International Livestock Research Institute), Nairobi, Kenya. pp. 125–132.
- Mukhebi A.W. 1996. Assessing economic impact of tick-borne diseases and their control: The case of theileriosis immunisation. In: Irvin A.D., McDermott J.J. and Perry B.D. (eds), *Epidemiology of Ticks and Tick-borne Diseases in Eastern Central and Southern Africa. Proceedings of a Workshop held in Harare, Zimbabwe, 12–13 March 1996*. ILRI (International Livestock Research Institute), Nairobi, Kenya. pp. 147–155.
- Perry B.D. 1996. Tick-borne disease control: The role of impact assessment. In: Irvin A.D., McDermott J.J. and Perry B.D. (eds), *Epidemiology of Ticks and Tick-borne Diseases in Eastern Central and Southern Africa. Proceedings of a Workshop held in Harare, Zimbabwe, 12–13 March 1996*. ILRI (International Livestock Research Institute), Nairobi, Kenya. pp. 139–146.

Summary of discussion: Socio-economics and impact assessment

Facilitator: A. Mukhebi

Rapporteurs: J. Mutugi and E. Peeler

In this session, the factors influencing the uptake, sustainability and impact of ECF immunisation, and the data required to assess these issues were described.

Tick control after ECF immunisation

Following immunisation tick control is required to control tick damage, reduce risk of ECF breakthroughs due to high challenge and to control other tick-borne diseases (TBDs). Where there is a history of intensive dipping and the presence of other TBDs, the potential risk of these diseases must be thoroughly considered before reducing tick control measures. Creating endemic stability by strategic dipping to allow the development of natural immunity or by immunisation may be necessary. Where there is a history of less intensive dipping, tick control can be reduced to threshold dipping when engorging ticks are detected, to limit tick infestation and tick damage. Experience in Uganda has shown that tick control can be relaxed safely by 50–60%, for example, from dipping twice to once a week. Acaricide is applied when engorging females are seen, and this helps to prevent the pasture being repopulated. *Boophilus* species are easily controlled by acaricide application every three weeks. However, if other TBDs are present, acaricide use may have to be intensified.

The question of what degree of reduction in acaricide use would ensure sufficient field challenge to boost ECF immunity following immunisation remains to be seen. It was generally felt that there is insufficient data to allow a general recommendation on this. In addition, factors such as ecological zone, cattle type and management system would affect the level of reduction. A pragmatic approach makes better sense, where a progressive reduction in acaricide use is made following immunisation until a safe minimum level is reached, suitable for a given circumstance. The issue of whether recommendations about tick control should appear on the immunisation product label was also raised. Participants felt that the farmer should be informed of the benefits and risks involved in immunisation to help him/her make a rational decision whether or not to adopt the technology. They concluded that there is insufficient information currently available on which to base recommendations. However, the view was expressed that, with the current state of knowledge, it should be possible to formulate recommendations on tick control for situations where *Babesia bigemina*, *B. bovis* or heartwater are present. Some participants felt the product should not be released until tick control recommendations were formulated.

Initially, the main target group for immunisation is farmers whose first priority is to reduce the risk of ECF, before reducing acaricide use. As the product becomes established in the market, some farmers will adopt the technology primarily to reduce the pattern of acaricide usage. In general, recommendations on reduced tick control will vary greatly between ecological zones, cattle types, management systems and tick challenge, complicating the effort to produce clear-cut guidelines. It was concluded that a pragmatic approach to reduced acaricide use was required, but that appropriate data is generated through controlled investigations to provide recommendations for varying conditions. Participants further noted that there was no way of managing the use of acaricides so that tick resistance is prevented.

Costs of vaccine and immunisation

Currently, the farm gate price for ECF immunisation ranges between US\$ 9 and 25 per adult cow. The cost largely depends on the clustering or density of cattle to be immunised and the use or non-use of a long-acting tetracycline formulation ('mild' vaccine strain, e.g. Boleni). Costs could be reduced in several areas including production, drug use and monitoring. The cost of treating reactors is built into the current price of the vaccine. As the number of reactors varies with different vaccines, batches of vaccine and cattle type, this may influence price greatly. When full cost recovery becomes a reality, the cost of treatment of reactors with buparvaquone may need to be separated from the vaccine cost. Monitoring costs could be significantly reduced if paraveterinarians and farmers are fully involved in monitoring, and if the number of visits is reduced or combined with complementary farm activities or services. Currently, some countries are making partial cost-recovery for ECF immunisation. However, in the long-term, there should be movement towards full cost-recovery or privatisation, in line with macro-economic policy reforms under structural adjustment programmes. It was pointed out that the ex-factory stabilate price was fairly easy to estimate accurately, and several estimates are available. The stabilate cost is a very small proportion of the final price to the farmer. It will vary a little depending on the number of doses available from each batch. In the production of Katete strain, fairly consistent batch sizes have been achieved. However, the farm-gate price of the stabilate will vary more depending on delivery systems (private or public) and transaction costs. In Zimbabwe, the overall price of tick control has been given to the farmer, which has meant the introduction of a combined immunisation and dipping levy.

Policy, legislation and legal issues

National policies should provide a framework to an integrated approach to the control of ticks and TBDs while also promoting the establishment of endemic stability. In addition, the policies should provide for a pattern of acaricide use that does not lead to the development of resistance or environmental pollution and animal product residues. The relevant legislation should also be revised to reflect the overall policy framework and include

the registration of vaccines. Legislation should also be extended to cover areas such as personnel administering the vaccine, classification of the vaccine, compensation and indemnity, and carrier state with respect to exporting cattle. There was general agreement that compensation and indemnity were unworkable. Instead, participants felt that the risk of reactors and breakthroughs should be described in the product data sheet and explained to the farmer. It was considered unrealistic to make immunisation compulsory, particularly as immunisation is a 'private good' for which beneficiaries are expected to pay.

Alternative vaccines

Subunit vaccines for the control of *Theileria parva*

A. Musoke, S. Morzaria and V. Nene

International Livestock Research Institute (ILRI),
P.O. Box 30709, Nairobi, Kenya

Introduction

Improvement of the livestock industry in many developing countries has been severely constrained by tick-borne diseases. The major protozoan pathogens responsible for these diseases in cattle are *Theileria parva* and *T. annulata* which cause East Coast fever (ECF) and tropical theileriosis, respectively, and *Babesia bigemina* and *B. bovis*, which cause redwater fever. The rickettsial organisms, *Cowdria ruminantium* and *Anaplasma marginale* are responsible for heartwater and anaplasmosis, respectively. East Coast fever is of great economic importance throughout eastern, central and southern Africa, with losses in 1989 estimated at US\$ 168 million (Mukhebi et al 1992). *Theileria annulata* is of even greater importance and occurs from North Africa to China (see review by Irvin and Morrison 1987). Though there are no recent figures for losses due to *T. annulata*, over 100 million cattle are at risk of contracting the disease. Babesiosis has a world-wide distribution. Anaplasmosis is also a disease with significant impact on the cattle industry throughout the world. It has been estimated that, in the USA alone, mortality approaches 100,000 cattle annually (Goodger et al 1979). Heartwater is widespread in sub-Saharan Africa and has been introduced to the Caribbean Islands (see review by Uilenberg 1993). The close proximity of these islands to the USA and South and Central America poses a threat to these countries. The disease ranks third in importance behind theileriosis and trypanosomosis in Africa but its economic importance has not been evaluated. All of the above tick-borne pathogens constitute a major impediment to the introduction of highly susceptible *Bos taurus* cattle into endemic areas and are the cause of high morbidity and mortality. This review will focus on recent research towards the development of a subunit vaccine against *T. parva*.

Current methods of control

The prevailing strategy of tick-borne disease control is based on the control of the tick vector using acaricides. This strategy is unreliable for a number of reasons including lack of funds for the purchase of acaricides, poor maintenance of control facilities (e.g. dips) and the development of acaricide resistance in tick populations. Additional concerns relate to environmental contamination. The major disadvantage of available vaccines against tick-borne diseases is that they are all based on the use of live organisms.

Currently, the only practical method of immunisation against ECF is infection with cryopreserved sporozoites and simultaneous treatment with long-acting formulations of oxytetracycline (Radley 1981). There are two major limitations to this method: firstly, because live organisms are used, a cold chain is required; secondly, protection is only ensured against the immunising stocks (Radley 1981). Attempts to develop a schizont-infected cell vaccine for *T. parva* were frustrated by the requirement for large numbers of cells to effect immunisation and observations of severe parasitosis in a proportion of cattle following inoculation (Brown et al 1978; Dolan et al 1982).

Progress towards development of a subunit vaccine

The approaches used to identify antigens for subunit vaccine development are, in general, guided by the type of immune responses that are likely to mediate protection. Two such immune mechanisms have been identified for *T. parva*. The immunity that protects the animal against the pathogenic schizont stage of this parasite is not dependent on antibody (Mohammed et al 1975). The development of techniques for *in vitro* transformation of bovine lymphocytes with *T. parva* and the maintenance of infected cell lines in culture (Brown et al 1973) were the most significant advances in allowing the elucidation of cellular immune mechanisms against the parasite. It was quickly demonstrated that immune peripheral blood lymphocytes proliferated in the presence of autologous infected cells, and that these cultures contained parasite-specific killing activity (Pearson et al 1979). The availability of a comprehensive range of bovine tissue typing sera allowed the demonstration that this activity was restricted by class I elements of the bovine MHC (Morrison et al 1987). It was also established that the effector cells were CD8⁺ T lymphocytes, which confirmed that cattle deploy classical cytolytic T lymphocytes against *T. parva* (Goddeeris et al 1986). Prominent parasite-specific CD4⁺ T cell responses have also been detected in immune cattle (Baldwin et al 1987; Brown et al 1989), although the contribution of these cells to immunity has not been established. Kinetic analyses of parasite-specific cytotoxic T lymphocytes (CTL) activity revealed that it is temporally associated with the clearance of parasitosis in immune cattle (Morrison et al 1987), which was consistent with its being involved in the resolution of infection. More direct evidence for the role of CTL in parasite clearance was provided by the observation that protection could be transferred between immune and naive monozygotic twin calves undergoing lethal challenge in the CD8⁺ fraction of responding efferent lymph (McKeever et al 1994).

Although humoral responses are not implicated in immunity of recovered cattle, animals do develop sporozoite neutralising antibodies after multiple exposure to the parasite. This observation gave rise to the belief that sporozoite surface antigens might constitute neutralising vaccine candidates. The techniques for the identification of antigens recognised by antibodies are well established and for this reason considerable progress has been made in determining the immunising potential of sporozoite antigens of *T. parva*.

Antisera from cattle in ECF-endemic areas are polyspecific and on immunoblots bind to several sporozoite molecules which include proteins of relative molecular weights (Mr) 105, 85, 67, 55, and 31, kilo-Daltons (kDa). Candidate vaccine antigens were identified from this

repertoire of sporozoite antigens by raising monoclonal antibodies (MAbs) that neutralised sporozoite infectivity (Musoke et al 1984; Dobbelaere et al 1984). The majority of the MAbs bound to a sporozoite stage-specific 67 kDa molecule (p67) that is expressed on the surface membrane of the parasite. DNA coding for p67 was identified by immunoscreening a lambda gt11 cDNA expression library derived from sporoblasts of the Muguga stock of *T. parva*. It was established that the gene encoding p67 is present in a single copy and is split into two exons by a 29 bp intron (Nene et al 1992).

An *Escherichia coli* recombinant fusion protein, NS1-p67, containing all 709 amino acid residues of p67 fused to the first 85 residues of NS1, a non-structural protein of influenza virus A, has been used in cattle immunisation experiments. A semi-purified preparation of NS1-p67 formulated in saponin was used to immunise 9 cattle. All cattle developed high titres of IgG antibodies that bound to native p67 on immunoblots and neutralised sporozoite infectivity (Musoke et al 1992). The immunised animals together with 9 unimmunised controls were challenged with a 70% lethal dose of a *T. parva* (Muguga) sporozoite stablate. Of the 9 immunised animals, 4 did not react to challenge and had no detectable parasitosis. Two cattle underwent a mild febrile reaction with transient schizont parasitosis. The remaining 3 immunised cattle and the 9 control animals all developed severe ECF (Musoke et al 1992). Hence, 6 of 9 cattle were immune to ECF. Current efforts are directed towards increasing the degree of protection by improving the immunisation scheme. Additional studies are focused on the immune mechanisms responsible for protection in these cattle, since immunity does not appear to correlate with antibody titres (Musoke et al 1992).

Immunity engendered by recombinant p67 is likely to be directed against the sporozoite stage of the parasite, since the antigen is not expressed by the subsequent stages. Antigenic diversity between different populations of *T. parva* is well recognised and is believed to be responsible for breakthrough infections in cattle immunised by infection and treatment (Radley 1981). An important feature of p67 that relates to its use as a vaccine is that its sequence is highly conserved among different isolates of *T. parva* and we have recently demonstrated that cattle immunised with recombinant p67 are protected against challenge with heterologous cattle-derived parasite (Nene et al 1996). However, the p67 gene sequence of buffalo-derived parasites contains two small regions of polymorphism (Nene V. and Gobright E. unpublished observations) and the impact of this finding, if any, on vaccine effectiveness is under investigation.

While attempts are being made to increase the level of protection achieved with recombinant p67, a vaccine that incorporates antigens of the schizont, the target of parasite-specific CTL, may provide more long-lasting immunity in the field. Two methods are currently available for identifying antigens that are expressed in the context of MHC class I molecules. The most direct approach is the isolation, fractionation and sequence analysis of peptides associated with MHC class I molecules. Once a peptide of defined sequence is shown to sensitise target cells to lysis by specific CTLs, then the gene encoding the peptide can be isolated using synthetic oligonucleotides as probes. The second approach depends on gene expression in a transfected target cell. This strategy uses cDNAs expressed in transiently transfected cells expressing appropriate MHC molecules, in combination with lytic or cytokine release assays. Both of these strategies have been successfully applied to the

identification of tumour antigens, and are applicable to the search for antigens of *T. parva* that provoke parasite-specific CTL responses. In terms of vaccine potential, ideally the schizont antigen should be conserved among different parasite stocks. Should this not be the case, a cocktail of antigens would be required to give as wide a cover as possible. In addition, because of the processing requirements of class I, MHC-restricted antigen presentation, incorporation of a schizont antigen component in recombinant vaccine against *T. parva* will require the use of appropriate antigen delivery systems such as those based on recombinant viruses or bacteria.

Concluding remarks

A successful recombinant tick-borne disease vaccine should ideally induce immunity, at least comparable to that engendered by available live vaccines in the degree of protection and longevity of immunity. The failure of recombinant of p67 to induce solid protection may result from the inability of the expression systems currently employed to make secondary modifications to the expressed products that are important for immune recognition.

A major consideration in designing vaccines for haemoparasites is that many of these organisms have at least two life cycle stages in the host animal. A successful vaccine may need to include antigens of different life cycle stages. For example, an effective vaccine for ECF might require sporozoite and schizont antigen components (Musoke et al 1992). Similarly, for tick-borne parasites where polymorphic antigens have been identified it may be useful to expand the search for conserved antigens expressed by other life cycle stages, in particular the tick derived stages.

As candidate vaccine antigens are identified, the identification of appropriate antigen delivery systems will become increasingly important. With the expansion of knowledge of the inductive requirements of the immune responses, delivery systems are available that engender the appropriate humoral or cellular immune responses. The choice of formulation is heavily influenced by the nature of the desired response. Additional criteria that must be applied relate to the infrastructure and financial capacity in developing countries. Most of the diseases under discussion are prevalent in countries where resources are limited. The live antigen delivery systems, based on recombinant viruses and bacteria, hold many advantages for these countries. Expression constructs can be manipulated to optimise the desired immune mechanisms, and, in theory at least, multiple antigen vaccines can be constructed with relative ease. More importantly, live antigen delivery vectors abrogate the need for production and purification of large quantities of antigen, and so are inherently less expensive. Current fears of environmental contamination with genetically engineered micro-organisms are likely to diminish with the development of highly attenuated vector strains coupled with successful field trials of existing live recombinant vaccines. For example, rabies vaccines based on recombinant pox viruses have now been deployed successfully in the field in both Europe and the USA (Brochier et al 1991; Pastoret and Brochier 1992).

References

- Baldwin C.L., Black S.J., Brown W.C., Conrad P.A., Goddeeris B.M., Kinuthia S.W., Lalor P.A., Machugh N.D., Morrison W.I., Morzaria S.P., Naessens J. and Newson J. 1987. Bovine T-cells, B-cells, and null cells are transformed by the protozoan parasite *Theileria parva*. *Infection and Immunity* 56:462-467.
- Brochier B., Kieny M.P., Costy F., Coppens P., Bauduin B., and Lecocq J.P. 1991. Large-scale eradication of rabies using recombinant vaccinia-rabies. *Nature* 354:520-522.
- Brown C.G.D., Crawford J.G., Kanhai G.K., Njuguna L.N. and Stagg D.A. 1978. Immunization of cattle against East Coast fever with lymphoblastoid cell lines infected and transformed by *Theileria parva*. In: Wilde J.K.H. (ed), *Tick-borne Diseases and their Vectors*. University of Edinburgh, Edinburgh, UK. pp. 331-333.
- Brown C.G.D., Stagg D.A., Purnell R.E., Kanhai G.K. and Payne R.C. 1973. Infection and transformation of bovine lymphoid cell *in vitro* by infective particles of *Theileria parva*. *Nature* 245:101-103.
- Brown W.C., Sugimoto C., Conrad P.A. and Grab D.J. 1989. Differential response of bovine T-cell lines to membrane and soluble antigens of *Theileria parva* schizont-infected cells. *Parasite Immunology* 11:567-583.
- Dobbelaere D.A.E., Spooner P.R., Barry W.C. and Irvin A.D. 1984. Monoclonal antibody neutralizes the sporozoite stage of different *Theileria parva* stocks. *Parasite Immunology* 6:361-370.
- Dolan T.T., Njuguna L.N. and Stagg D.A. 1982. The response of *Bos taurus* and *Bos indicus* cattle types to inoculation of lymphoblastoid cell lines infected with *Theileria parva* schizonts. *Tropenmedical Parasitology* 33:57-62.
- Goddeeris B.M., Morrison W.I., Teale A.J., Bensaid A. and Baldwin C.L. 1986. Bovine cytotoxic T-cell clones specific for cells infected with the protozoan parasite *Theileria parva*: Parasite strain specificity and class I major histocompatibility complex restriction. *Proceedings of the National Academy of Sciences USA* 83:5238-5242.
- Goodger W.J., Carpenter T. and Riemann H. 1979. Estimation of economic loss associated with anaplasmosis in California beef cattle. *Journal of American Veterinary Medicine Association* 174:1333-1336.
- Irvin A.D. and Morrison W.I. 1987. Immunopathology, immunology and immunoprophylaxis of *Theileria* infections. In: Soulsby E.J.L. (ed), *Immune Responses in Parasitic Infections: Immunopathology and Immunoprophylaxis*. CRC Press, Boca Raton, USA. pp. 233-274.
- McKeever D.J., Taracha E.L.N., Innes E.A., MacHugh N.D., Awino E., Goddeeris B.M. and Morrison W.I. 1994. Adoptive transfer of immunity to *Theileria parva* in the CD8-fraction of responding efferent lymph. *Proceedings of National Academy Sciences USA* 91:1959-1963.
- Morrison W.I., Goddeeris B.M., Teale A.J., Grocock C.M., Kemp S.J. and Stagg D.A. 1987. Cytotoxic T-cells elicited in cattle challenged with *Theileria parva* (Muguga): Evidence for restriction by class I MHC determinant and parasite-strain specificity. *Parasite Immunology* 9:563-578.
- Mohammed S.I., Lauerman L.H. and Johnson L.W. 1975. Effect of humoral antibodies on the course of *Theileria parva* (East Coast fever) of cattle. *American Journal of Veterinary Research* 36:399-402.
- Mukhebi A.W., Perry B.D. and Kruska R. 1992. Estimated economics of Theileriosis control in Africa. *Preventive Veterinary Medicine* 12:73-85.
- Musoke A.J., Morzaria S.P., Nkongse C.G., Jones E. and Nene V. 1992. A recombinant sporozoite surface antigen of *Theileria parva* induces protection in cattle. *Proceedings of the National Academy of Science USA* 89:514-518.
- Musoke A.J., Nantulya V.M., Rurangirwa F.R. and Buscher G. 1984. Evidence for a common protective antigen determinant on sporozoites of several *Theileria parva* strains. *Immunology* 52:231-238.

- Nene V., Iams K.P., Gobright E. and Musoke A.J. 1992. Characterisation of the gene encoding a candidate vaccine antigen of *Theileria parva* sporozoites. *Molecular and Biochemical Parasitology* 15:17-28.
- Nene V., Musoke A., Gobright E. and Morzaria S. 1996. Conservation of the sporozoite p67 vaccine antigen in cattle-derived *Theileria parva* stocks with different cross-immunity profiles. *Infection and Immunity* 64:2056-2061.
- Pastoret P.P. and Brochier B. 1992. Development of a recombinant vaccines-rabies vaccine for oral vaccination of foxes against rabies. *Developments in Biological Standardization* 79:105-111.
- Pearson T.W. , Lundin L.B., Dolan T.T. and Stagg D.A. 1979. Cell-mediated immunity to *Theileria* transformed cell lines. *Nature* 281:678-680.
- Radley D.E. 1981. Infection and treatment method of immunisation against theileriosis. In: A.D. Irvin, M.P. Cunningham, A.S. Young (eds), *Advances in the control of theileriosis*. Martinus Nijhoff, The Hague, The Netherlands. pp. 227-237.
- Uilenberg G. 1983. Heartwater (*Cowdria ruminantium*) infection: Current status. *Science and Comparative Medicine* 27:427-480.

Multi-component subunit vaccines against *Theileria parva*

D. McKeever

International Livestock Research Institute (ILRI),
P.O. Box 30709, Nairobi, Kenya

Background

Cattle that recover from *Theileria parva* infection are solidly immune to rechallenge with homologous parasites. Most of the International Laboratory Research on Animal Diseases'/International Livestock Research Institute's (ILRAD/ILRI) immunological research into the disease has focused on defining the basis of this protection. Over 15 years of basic and applied immunological research at ILRAD/ILRI have demonstrated that protection in *T. parva*-immune cattle is primarily mediated by parasite-specific cytotoxic T lymphocytes (CTL). Available evidence suggests that these responses require the input of specific helper cells and endure for the life of the animal. Although CTL immunity can be induced by infection of cattle in the face of tetracycline treatment, for a number of reasons this method is not considered sustainable. Development of a subunit vaccine for generating parasite-specific CTL has therefore, been a major focus of the East Coast fever (ECF) vaccine development programme for several years.

Status

Research has focused on three areas:

- identifying *T. parva* antigens recognised by bovine CTL and associated helper cells
- understanding how bovine CTL responses are activated *in vivo*
- identifying antigen delivery systems capable of generating specific CTL responses in cattle.

Options

Antigens

The two major strategies that have been reported for identification of CTL antigens involve complex technologies known as peptide stripping and COS cell screening. Both approaches are well under way at ILRI and are supported by collaboration with appropriate leading

research institutes. An additional opportunity arises from a recently initiated Japanese-funded *T. parva* genome-sequencing project at ILRI. Antigens recognised by helper cell populations can be identified by more traditional methods in which ILRI has much experience.

***In vivo* activation requirements**

ILRI has substantial expertise in dissecting cellular immune mechanisms and is focused on the interactions between CTL and associated helper cells.

Antigen delivery systems

A number of systems have been reported to provoke CTL responses *in vivo*, among which the use of live recombinant micro-organisms has proved most consistently effective. In view of their potential for use under conditions where ECF is endemic, ILRI research has focused largely on the evaluation of recombinant viruses, bacteria and naked DNA vectors.

Training requirements for vaccine delivery

ECF immunisation: Training requirements for veterinarians and field staff

R. Rumberia

National Veterinary Research Centre (NVRC), Muguga,
P.O. Box 32, Kikuyu, Kenya

Introduction

Over the years, various methods of immunisation have been used in an effort to protect cattle against East Coast fever (ECF) (Purnell 1977). However, only immunisation by infection and treatment has so far proven suitable. The method involves inoculation of live *Theileria parva* sporozoites and concomitant treatment with tetracyclines (Radley 1981). For a number of reasons, there has been some reluctance to adopt infection and treatment (ITM) as a control method against ECF. These include the lack of a single vaccine stock that will protect against a wide range of heterologous parasites, the fear of introducing new parasites to areas where they do not exist, concern about the carrier state and the effect of immunisation on productivity. Most of these concerns have been recognised and studies have been undertaken to address them. For example, Radley et al (1975) explored the possibility of using a combination of three *T. parva* stocks and showed that they could protect cattle against a range of parasites far removed from the source of immunising strains. This parasite combination, referred to as the 'Muguga Cocktail', is used in parts of eastern, central and southern Africa for immunisation. Mutugi et al (1990a) showed that a single isolate (*T. parva* Marikebuni) protected against 28 *T. parva* isolates from widely scattered regions of Kenya. This stock is currently the recommended material for immunisation in Kenya. Conclusive evidence has been presented showing that animals that recover from ECF remain carriers (Young 1981; Kariuki 1991) thus removing earlier concerns on the role of carrier status in the epidemiology of the disease. Other studies have revealed that immunisation has no effect on productivity (Mutugi et al 1990b) or fertility (Dolan and Mutugi 1989; Rumberia et al 1993). One of the major problems associated with the infection and treatment method against ECF is the possibility of creating clinical reactions (reactors) due to the use of pathogenic vaccine material, which, unlike most other animal vaccines, neither attenuated nor killed. This is not unique for ECF since other diseases, like heartwater, employ a similar approach.

Training

Why is training necessary?

There are two main reasons for providing training on ECF immunisation. Firstly, veterinarians and their assistants need to update their knowledge on the disease to help

accurately assess the success or failure of the vaccine. Although considerable literature exists on the causative agent, the epidemiology of the disease, immune mechanisms and control, this knowledge may not be readily available to practitioners resulting in misunderstanding of the efficacy and safety of the vaccine. Secondly, since the ITM is based on live and pathogenic material, the method involves follow-up monitoring of immunised animals for reactions. Professionals and their assistants involved in the delivery system thus need to be trained in handling the vaccine and its delivery and in identifying and treating reactors. Over the last three years, the National Veterinary Research Centre (NVRC) has carried out 5 training sessions involving a total of 49 veterinarians and 56 technical health personnel in Kenya. It was evident that there was a serious need for theoretical and practical training for veterinarians and support personnel involved in the delivery system to help successfully deliver the technology. This may also be true in other countries where ITM is being practised. The ITM is a relatively new technology and farmers also need basic information on the nature, delivery and the associated risks and advantages of the exercise.

Who should be trained?

The policy in Kenya provides that immunisation will be carried out privately either by veterinarians in private practice or in government service in their private capacity. Although veterinarians should be the primary target group for training, there is also need to train technical personnel (paraveterinarians and laboratory technicians) who may assist during the immunisation and laboratory back-up and agricultural extension personnel who will help sensitise farmers on the technology. Students at tertiary level institutions (university and colleges) could also be potential targets to help reach a wider audience. One of the major constraints of the method is the need for close monitoring of animals after immunisation. Training of farmers and their workers in monitoring for reactors would thus substantially contribute to reducing the cost of immunisation and help make it more affordable.

Training curriculum

The training should focus on both the theoretical and practical aspects of the disease, tackling issues associated with vaccine delivery and post-immunisation monitoring. It is envisaged that, in the long term, the trained veterinarians will become trainers of technical-support and extension staff and farmers. The course content may vary depending on the role played by the trainees in the delivery system. For example, veterinarians need more detailed theoretical training sessions than the technical staff although both will require similar practical exposure.

In Kenya, a training manual developed by Mbogo et al (1995) has been used for training. The theoretical session covers such issues as the aetiology, life cycle of the parasite in the bovine and the tick vector, transmission, clinical signs, pathology and immunity and the basics of infection and treatment. Other tick-borne diseases (anaplasmosis, babesiosis and heartwater) are also covered in a similar format but in less detail. A series of questions follow each section to evaluate the trainees understanding of the issues. Practical sessions include preparation and handling of stabilates, immunisation and monitoring. Practicals consist of

demonstrations as well as participation by the trainees. In addition to group training, project staff work closely with the trained personnel during their first immunisation projects as a way of building their competence and confidence.

The programme has yet to be evaluated. Some of the considerations include the course content for the different groups, course duration and training of the trainers. Currently, the trainers are scientists who may not have adequate communication skills to sufficiently fulfil their role as trainers.

Training venues

Due to the nature of the vaccine, training in ECF immunisation must be thorough. The available human and physical resources to be used for the training must be clearly identified. The various categories of trainees involved in the delivery system require different facilities. Rooms with adequate facilities will be needed for teaching the theoretical aspects of the disease for all cadres. Simpler facilities like open grounds may be adequate during sensitisation of farmers and extension staff in the form of barazas or field days. Practical training will require laboratories and suitable animal facilities. Ideally, the theoretical and practical training should be done where the technology and expertise for the ITM exist. Such facilities may be found in international (e.g. International Livestock Research Institute (ILRI)), regional (e.g. Vaccine Production Centre (VPC), Malawi) or national (e.g. NVRC, Kenya Agricultural Research Institute –(KARI)) institutions. Training may also be moved to other centres with adequate facilities like veterinary investigation laboratories, farmer training institutes or tertiary institutions. From the classroom, training should be extended to become on farm where the trainees will work alongside the trainers during all phases. This is essential due to the close individual contact between the trainers and trainees. Farmers can also be trained, particularly in clinical monitoring, if a decision has been made to involve them in the process. Other avenues like radio, farmers' bulletins, the press and leaflets may also be used to reach and sensitise the farmers to ECF immunisation.

Funding

Training sessions require financial inputs like remuneration of trainers, hiring of facilities and training material. Funding could be obtained from donor agencies, non-governmental organisations (NGOs), governments or the private sector particularly those involved in the sale of the vaccine.

References

- Dolan T.T. and Mutugi J.J. 1989. The fertility of Boran heifers immunised against buffalo-derived *Theileria parva*. In: Dolan T.T. (ed), *Theileriosis in Eastern, Central and Southern Africa. Proceedings of a Workshop on East Coast Fever Immunization, Lilongwe, Malawi, 20–22 September 1988*. ILRAD (International Laboratory for Research on Animal Diseases), Nairobi, Kenya. pp. 68–70.,

- Kariuki D.P. 1991. *Studies on the Carrier State of East Coast Fever (T. parva) in Relation to the Epidemiology and Control of the Disease*. PhD thesis, University of Salford, Salford, UK.
- Mbogo S.K., Kariuki D.P., McHardy N. and Payne R. 1995. *Training Manual for Veterinary Staff on Immunisation against East Coast Fever Using the ECFiM System*. KARI (Kenya Agriculture Research Institute), Nairobi, Kenya.
- Mutugi J.J., Young A.S., Linyonyi A., Mining S.K., Maritim A.C., Ngumi P.N., Lesan A.C., Stagg D.A., Ndungu S.G. and Leitch B.L. 1990a. Problems associated with identification of protective *Theileria parva* stocks for immunisation. In: Young A.S., Mutugi J.J. and Maritim A.C. (eds), *Progress Towards the Control of East Coast Fever (theileriosis) in Kenya*. (KARI) Kenya Agriculture Research Institute, Nairobi, Kenya. pp. 40–48.
- Mutugi J.J., Young A.S., Ndungu S.G., Linyonyi A., Maritim A.C., Mining S.K., Ngumi P.N., Kariuki D.P., Leitch B.L., de Souza D., Maloo S. and Lohr K.P. 1990b. Response to a vaccine trial for East Coast fever in two herds at the Kenya coast. *Preventive Veterinary Medicine* 10:173–183.
- Purnell R.E. 1977. East Coast fever of cattle: Some recent research in East Africa *Theileria*. *Advances in Parasitology* 15:83–132.
- Radley D.E., Brown C.G.D., Cunningham M.P., Kimber C.D., Musisi F.L., Payne R.C., Purnell R.E., Stagg D.A. and Young A.S. 1975. East Coast fever: 3. Chemoprophylactic immunisation of cattle using oxytetracycline and a combination of *Theileria* strains. *Veterinary Parasitology* 1:51–60.
- Radley D.E. 1981. Infection and treatment method of immunization against theileriosis. In: Irvin A.D., Cunningham M.P., and Young A.S. (eds), *Advances in the Control of Theileriosis. Proceedings of an International Conference held at the International Laboratory on Animal Diseases, Nairobi, Kenya, 9–13 February 1981*. Current Topics in Veterinary Medicine and Animal Science Volume 14. The Hague, the Netherlands, Martinus Nijhoff, pp. 227–237.
- Rumberia R.M., Rowland A.C., Watson E.D., Eley R.M. and Young A.S. 1993. The effect of immunisation against theileriosis on the reproductive function of Boran/Friesian cross heifers—a preliminary study. *British Veterinary Journal* 150:301–303.
- Young A.S. 1981. The epidemiology of theileriosis in East Africa. In: Irvin A.D., Cunningham M.P. and Young A.S. (eds), *Advances in the Control of Theileriosis. Proceedings of an International Conference held at the International Laboratory on Animal Diseases, Nairobi, Kenya, 9–13 February 1981*. Current Topics in Veterinary Medicine and Animal Science Volume 14. Martinus Nijhoff Publishers, The Hague, The Netherlands. pp. 38–55.

Extension programme development for the delivery of East Coast fever (ECF) vaccines

M. Moran

Food and Agriculture Organization of the United Nations (FAO), Representative Office,
P.O. Box 521, Kampala, Uganda

General

Extension can be defined as the method of transmitting a specific message or technology to a particular target group of beneficiaries.

Target population (beneficiaries)

The group of people to benefit from the new technology should be carefully identified. Their perception and understanding of the subject matter require evaluation.

Extension theme (message)

The message of a particular extension programme should remain as simple and straightforward as possible. All attempts should be made to avoid controversial issues that would only create confusion. Some messages are designed to provide specific information while others may be required to help convince a particular beneficiary group that a problem actually exists and that there is a new technology available to improve the situation.

Communication

This is the channel through which the extension theme is transmitted to the target population. A number of channels could be used to transmit messages but the most appropriate means to get the information across should be selected. Some examples are:

- Verbal: farmer meetings and demonstrations
- Written: explanatory handouts, brochures, posters, animated stories, technical manuals, newspaper articles etc
- Audio: radio messages and cassette recordings
- Audio-visual: flipchart programmes, slide or filmstrip presentations, videocassette, television etc.

Most extension campaigns combine several communication methods in an attempt to provide maximum exposure to the target group.

Extension development to promote ECF vaccine delivery

Target population

In most situations, this will be farmers attempting to increase production by introducing improved cattle breeds (both dairy and beef).

Extension theme

An alternative approach to ECF control is available which includes the use of ECF vaccines coupled with relaxed tick control strategies. The message should provide information covering the target population of cattle, immunisation procedure, post-immunisation monitoring requirements, benefits, potential risks involved and the total cost of vaccine delivery. Most of the target population (beneficiary group) thoroughly understands the economic impact of ECF and intensive tick control. Therefore, the message probably does not require a detailed discussion on the negative impact of ECF disease in improved cattle. The message should focus attention on this alternative approach to ECF control.

Communication

This will be highly variable depending on the resources available and will involve a variety of communication techniques.

It should be noted that extension campaigns should be developed to support vaccine delivery after the services have been adequately institutionalised.

Recommendations

Recommendations

General

1. The participants agreed to compile and circulate a summary of recommendations from all previous meetings, including those under the regional programme since 1984. This summary of recommendations should be made available during future meetings, which could begin by reviewing potential follow-up activities and evaluate progress made.
2. Members of the technical training manual and farmer brochure production groups should be identified from different country projects. These groups should meet and continue with the preparation of standard material for use at the regional level. The literature produced must also be adaptable to local requirements, particularly with reference to language.
3. The region should move towards establishing a central (regional) vaccine production laboratory that provides standard vaccine stabilates to national projects. These stabilates must be prepared according to agreed minimum standards and pass through defined quality control criteria.
4. It is vital that a repository (or repositories) is created to store key immunising stocks for the region and also important reagents related to vaccine deployment and characterisation.
5. There is an urgent need to formulate post-immunisation tick control strategies using already existing knowledge. Information describing the various strategies and advice on how to select the most appropriate for a particular situation must be made available to field veterinarians and cattle owners.
6. The regulatory and monitoring capacity of governments should be strengthened as the technology of immunisation moves toward the private sector. This would assist issues, such as, the licensing of both vaccines and personnel involved in immunisation.
8. Institute a working group responsible to prepare an action plan for the training requirements of the region with particular emphasis on vaccine delivery. In some areas, skilled veterinarians and animal health assistants are not currently available for training in vaccine delivery. The question of fair distribution of trained personnel in these areas, which is primarily a funding issue, must be addressed.
7. Previous meetings have recommended that a minimal data set be formulated which would identify the minimum information required to give useful post-immunisation surveillance. This has not yet been done and the recommendation was reiterated at this meeting.

9. An action group should be set up to help find the means to retain trained national staff. This group should identify the various fundamental issues involved in loss of staff and recommend possible solutions. There has been some success in South America and Asia where mechanisms have been developed to help retain trained national staff.
10. The region should be proactive to obtain additional funding. It should formulate an action plan for the control of ticks and tick-borne diseases (TBDs) to target at donors, particularly donors who have not previously provided funding for the region.

Research

1. More information is required from different epidemiological zones, where different combinations of TBD occur, to help recommend appropriate integrated tick and TBD control strategy following immunisation.
2. Post-immunisation surveillance is essential for several reasons:
 - To continue to monitor possible breakthroughs in the field. Isolation, characterisation and cross-immunity evaluation of breakthrough parasites is desirable, but is not logistically or financially feasible. This makes pursuing a rational approach to the investigation of possible breakthroughs more important.
 - To assist in assessing the relative benefits of the different tick control strategies adopted after immunisation in different epidemiological zones.
 - To help impact assessment studies determine the effects of immunisation (such studies require pre-immunisation data to determine the impact of ECF before immunisation).
 - To look at the impact of immunisation on the productivity of the livestock industry and the epidemiology of ECF.
3. Each vaccine stock in use requires a unique marker to differentiate it from other vaccines and field parasites. These markers are likely to be the products of current research at the International Livestock Research Institute (ILRI).
4. Studies are required to determine the earliest age at which calves can be immunised safely and effectively.
5. The search for mild strains of *Theileria parva*, which could be used for vaccination without oxytetracycline, should continue.
6. Complications resulting from the use of levamisole together with available vaccines should be a research area of high priority.
7. Cross-immunity trials between vaccine stocks in use (Muguga cocktail, Marikebuni, Boleni and Katete) should be a top priority in ECF vaccine development.
8. The vaccine diluent needs to be improved to make it more user-friendly with less pH lability and, if possible, without the -20°C storage requirement.

9. Policy research relating to TBD immunisation is important, as the current policies in many countries of the region need updating to accommodate the principles of integrated control. Examples include the cattle cleansing act and the fact that ECF is a notifiable disease in some countries.

Summary of recommendations from Regional Tick and Tick-borne Disease Control Workshops held between 1976 and 1997

T. Dolan

Livestock Services, P.O. Box 24720, Nairobi, Kenya

The regional workshop in March 1997 agreed that the recommendations made at all previous workshops in this series should be compiled and circulated. Participants at this workshop felt that the process of making recommendations was becoming more an exercise in wishful thinking than one likely to bring about the actions required. Having a summary of the recommendations would thus serve as an easy reference that could be updated or amended. It could also be used to assess the importance or priority given to the recommendations and review the actions taken.

The workshop participants recommended that the summary be limited to the recommendations of the regional workshops in this series, held in 1984 (Nairobi, Kenya), 1985 (Nairobi), 1988 (Lilongwe, Malawi), 1991 (Kampala, Uganda), 1994 (Lilongwe) and this one (Nairobi, 1997). However, a workshop sponsored by the International Development Research Centre (IDRC) was held in Nairobi in 1976. Eight countries from the region were represented, together with the Organization of African Unity (OAU) and the International Livestock Research Institute (ILRAD/ILRI), and specific recommendations were made on *Theileria parva* and tick control. In addition, a workshop was held on the epidemiology of ticks and tick-borne diseases in the region in Harare in 1996, co-sponsored by the Food and Agricultural Organization of the United Nations (FAO) and ILRI at which 10 countries were represented. The recommendations from these two workshops have also been incorporated to the summary.

The summary highlights only those issues for which specific recommendations were made. They are summarised in (Tables 1–14) with *Theileria parva* as the focal point for control, particularly by infection and treatment immunisation, and with ticks and other *Theileria* species and tick-borne disease parasites categorised by each of the workshops. The tables contain topic headings from the workshops and the individual recommendations are included to provide a consistent linking across the different meetings. In some instances it has been necessary to interpret the intention of the recommendation to maintain the consistency of the format, while others have been included under a simple heading based upon the perceived intention. Recommendations might also appear in more than one table. This was unavoidable because the nature of some issues has changed between meetings necessitating a more specific recommendation. An example of this is data management under (vii) Training, (x) Epidemiology and (xi) Impact and productivity. Suggested research topics have also been presented as elements within the appropriate table.

The recommendations from regional workshops from 1976 have been extracted and compiled on diskette and in hard copy for future reference. These sets of information will be

lodged with the Programme Steering Committee through OAU for reference at future workshops and at ILRI.

Summary tables of recommendations made by Regional Workshops 1976-97

Table 1. *Acaricides and vector control.*

Topics	1976	1984	1985	1988	1991	1994	1996	1997
Tick counts and identification			+					
Management training (dipping)	+							
Resistance testing	+							
Cost/benefit analysis		+					+	+
Strategic/threshold application		+	+				+	+
Cattle movement control	+							
Biological/novel control	+		+					
Wild animal hosts of tick species	+	+						
Financial support	+							+

Table 2. *Immunisation: infection and treatment method.*

Topics	1976	1984	1985	1988	1991	1994	1996	1997
Investigate efficacy	+	+	+				+	
Implement		+	+				+	
Use three component vaccine		+	+					
Use local isolate vaccine		+	+					
Attention to risk of introduction of parasites† through vaccination				+		+	+	
Investigate breakdown breakthrough parasites		+	+				+	+
Produce vaccines centrally		+			+		+	+
Explore buffalo-derived parasites		+						
Investigate earliest age at which to vaccinate								+
Conduct cross-immunity among major immunising stocks						+		+

† unrelated and potentially pathogenic *Theileria parva*.

* as pre-investment phase.

Table 3. *Immunisation: new and improved vaccines.*

Topics	1976	1984	1985	1988	1991	1994	1996	1997
Tissue culture	+							
Different parasite stages	+							
Other blocking agents		+		+				
Improve diluent								+
Mild stocks								+
Antigenic variation of vaccine and buffalo-derived stocks		+						
Novel antigens		+						
Examine effect of levamisole								+
Other TBDs		+	+					

TBDs = tick-borne diseases.

Table 4. *Characterisation of parasites.*

Topics	1976	1984	1985	1988	1991	1994	1996	1997
Stock/strain	+	+	+	+	+	+	+	
Buffalo-derived		+						
Carrier		+					+	
Field and immunising				+	+	+		+

Table 5. *Safety and vaccine standards.*

Topics	1976	1984	1985	1988	1991	1994	1996	1997
Safety testing for extraneous agents			+	+				
Safety for staff handling ticks				+				
Thawing and long-term storage			+					
Infection rates in ticks			+					
Formal standards for vaccines				+	+	+		+
Deterioration of stabilates over time			+					
Reference stabilates of critical stocks		+	+					
Repository laboratories for stabilates and reagents		+						+
Storage and labelling				+				
Licensing and regulation							+	+
Funding	+							+

Table 6. *Chemotherapy.*

Topic	1976	1984	1985	1988	1991	1994	1996	1997
New drugs	+							

Table 7. *Standardised diagnostic tests.*

Topic	1976	1984	1985	1988	1991	1994	1996	1997
Major TBDs		+				+	+	

TBDs = tick-borne diseases.

Table 8. *Training.*

Topics	1976	1984	1985	1988	1991	1994	1996	1997
TBD control	+			+	+		+	+
Immunisation		+	+				+	+
Data management		+					+	
Impact assessment							+	

TBD = tick-borne disease.

Table 9. *Definitions and nomenclature.*

Topic	1976	1984	1985	1988	1991	1994	1996	1997
Definitions/terms and nomenclature		+		+	+			

Table 10. *Integrated strategies.*

Topics	1976	1984	1985	1988	1991	1994	1996	1997
Optimal integrated control			+				+	+
Policy research on legislation								+
Improve and harmonise technical and farmer manuals	+							+

Table 11. *Epidemiology.*

Topics	1976	1984	1985	1988	1991	1994	1996	1997
Parasite identification and characterisation	+			+	+	+		
Survey TBDs before immunisation and relaxation of tick control			+				+	+
Define important Theileria diseases			+					
Investigate (i) natural carrier state and (ii) following immunisation			+	+				
Monitor evolving parasite populations following immunisation				+			+	
Investigate changing epidemiology of TBDs through control				+	+			
Study chronic disease			+					
Study tick ecology			+					
Structured data sets and management			+	+	+		+	+
Model construction and use				+		+		
Decision support systems						+	+	

TBDs = tick-borne diseases.

Table 12. *Impact and productivity.*

Topics	1976	1984	1985	1988	1991	1994	1996	1997
Standardised procedure for vaccine delivery			+				+	+
Assess productivity following immunisation		+	+	+			+	+
Standardised data sets		+	+	+		+	+	+
Standardised methods for impact assessment			+	+		+	+	+
Precision in measurement of parameters			+				+	
Feedback on efficacy of control			+				+	
Central design and analysis of data			+			+		
Financial support							+	

Table 13. *Regional programme and co-ordination.*

Topics	1976	1984	1985	1988	1991	1994	1996	1997
Support regional programme			+	+	+	+	+	
Ensure national programme management			+	+			+	+
Develop delivery strategies				+			+	
Support for applied research	+						+	
Conduct impact assessment							+	
Retain trained national staff		+		+				+
Prepare summary of recommendations								+
Ensure donor support	+			+				+

Table 14. *Networks and information exchange.*

Topics	1976	1984	1985	1988	1991	1994	1996	1997
Information exchange/networks	+	+		+	+	+	+	
Regular workshops		+		+				
Regional programme role		+			+		+	
Training	+	+						

Comment

In reviewing the recommendations, it became apparent that many of them were repeated and, even when action had been taken on a particular recommendation, it might be repeated again. Thus, the summary will be important both for rationalisation of the recommendations and for planning and organisation of the Regional Programme. It should become a responsibility of the Regional Programme, either of the co-ordinator or of the Centre, to keep track of these recommendations and possible actions to be taken to address them. It might also be worthwhile to have 'action on the recommendations' as an item on the agenda for the Programme Steering Committee (PSC) to help keep up the momentum. The follow-up should, however, not be left to subsequent regional workshops, usually irregular and held at two- to three-year intervals. The summary of the recommendations should provide an opportunity for the participants at the regional workshop to gauge the level of progress of their inputs since they last met.

The changing nature of the Regional Programme and the transfer of ownership to the countries of the region (formally in June 1997 with OAU/IBAR as the executing agency) means that the countries themselves will be responsible, through the PSC, for ensuring that recommendations are acted upon. Thus, the regional workshop will no longer ask for donor support through an agency, it has direct access to the donor and responsibility for the programme through its own PSC.

Although recommendations have been repeated over the years, as if nothing had happened, progress has been made. For instance, the meeting of 1976 urged continued work on chemotherapy (which was showing promise at the time) and three effective compounds are now on the market. Standards have since been developed for vaccines (recommended in 1988 and 1991) and these regional standards largely determine those adopted by the Office International des Epizooties (OIE) (see OIE Manual of Standards for Diagnostic Tests and Vaccines, 1996, Theileriosis, pp. 321–330). The adoption of a standard terminology has also been achieved as recommended by the meeting of 1988, based on those of Irvin et al (1983, *Research in Veterinary Science* 35:341–346). In addition, the nomenclature issues that were being debated in 1988 were brought to the attention of the International Commission on Zoological Nomenclature. The Commission agreed that the proposed nomenclature was acceptable and that the expertise in the region was competent to determine appropriate nomenclature in keeping with the systems being adopted internationally.

The workshop in September 1984 on the collection, handling and analysis of performance and productivity data was the most specific of all the regional workshops, and while its intention was worthwhile, it did not achieve its objective. Much of what was intended from that workshop was repeated in an abbreviated form in the recommendations in 1994, and in considerable detail by the epidemiology workshop in 1996. It is hoped that with the new programme at the Centre for Ticks and Tick-borne Diseases (formerly the Vaccine Production Centre, Lilongwe, Malawi), with much of its emphasis on epidemiology training for the region, these most necessary of recommendations will become realities.

Occasionally a recommendation has been made which was not universally acceptable and which was revoked or modified later. An example is the recommendation from 1984

that a central, computerised data storage and software development system be set up. Similarly, in 1994 the rider that results must be fed back to participating countries and that the data remain the property of the country is yet another. The original recommendation recognised the confidentiality of such data but failed to clearly establish the question of subsequent ownership that undermined follow-up action. It is therefore important to clarify both the intention and the wording of issues in future recommendations.

Finally, recommendations on networks and information exchange have been made at six of the eight workshops and despite action being taken, those directly involved in tick-borne disease control do not seem to be receiving information and the recommendation is repeated. The regional workshops are being held and the proceedings have been and are being produced, the Vaccine Production Centre produced and circulated a useful newsletter and, more recently, the University of Florida/United States Agency for International Development (USAID)/Southern African Development Co-operation (SADC) Heartwater project has been producing its newsletter, *The Tickler*, which has been widely circulated, although it is not strictly a regional programme document. It was recognised in the discussions at some of the workshops that proceedings and other information may be reaching the upper echelons of the national programmes but not finding their way down to those actually doing the work. Ensuring the effective flow of information will be an important issue for the new Regional Programme to tackle.

List of participants

R. Bishop

ILRI

P.O. Box 30709, Nairobi, Kenya

Tel: +254-2-630743

Fax: +254-2-631499

Email: r.bishop@cgiar.org

P. Capstick

P.O. Box 16526

Nairobi, Kenya

S. Chema

AGREF

P.O. Box 39189, Nairobi, Kenya

Tel: +254-2-440832/443752

Fax: +254-2-448006

Email: agref@insightkenya.com

C. Chizonda

Animal Health and Industry

P.O. Box 2096, Lilongwe, Malawi

Tel: +265-744455/744313

Fax: +265-740323/742939

J. de Castro

FAO,

Via delle terme di Caracalla

00100 Rome, Italy

Tel: +39-6-52253634

Fax: +39-6-52255749

Email: julio.deCastro@field.fao.org

D. de Waal

ARC Onderstepoort Veterinary Institute

Private Bag x05

Onderstepoort 0110, South Africa

Tel: +27-12-529-9212

Fax: +27-12-529-9434

Email: theo@moon.oivi.ac.za

C. Di Giulio

P.O. Box 1068, Arusha, Tanzania

Tel: +255-57-8670

Fax: +255-57-8670

Email: lynenrpd@habari.co.tz

T. Dolan

Livestock Services

P.O. Box 24720, Nairobi, Kenya

Tel: +254-2-882003

Fax: +254-2-882456

Email: analabs@net2000ke.com

M. Finikiso

SADC Livestock Sector

P/Bag 0032, Gaborone, Botswana

Email: dahp@info.bw

A. Irvin

ILRI

P.O. Box 30709, Nairobi, Kenya

Tel: +254-2-630743

Fax: +254-2631499

Email: a.irvin@cgiar.org

G. Kaaya

ICIPE

P.O. Box 30772, Nairobi, Kenya

Tel: +254-2-861248

Fax: +254-2-860110

Email: icipe@cgnet.com

M. Kalunda

NARO

P.O. Box 295, Entebbe, Uganda

Tel: +256-42-20264

Fax: +256-42-21070/21126

D. Kariuki

NVRC-Muguga

P.O. Box 32, Kikuyu, Kenya

Tel: +254-154-32450

Fax: +254-154-884361

H. Kiara

NVRC-Muguga

P.O. Box 32, Kikuyu, Kenya

Tel: +254-154-32703

Fax: +254-154-884361

J. Leneman

FAO
Via delle terme di Caracalla
00100 Rome, Italy
Tel: +39-5-52253636
Fax: +39-6-52255749
Email: marjon.leneman@fao.org

L. Lynen

Veterinary Investigation Centre
P.O. Box 1068, Arusha, Tanzania
Tel: +254-57-3431/2
Fax: +255-57-8670
Email: lynenrdo@habari.co.tz

W. Madzima

Veterinary Services
P.O. Box CY 66
Causeway, Harare, Zimbabwe
Tel: +263-4-700327
Fax: +263-4-720879
Email: wmadzima@healthnet.zw

K. Majaliwa

Livestock Development
P.O. Box 9152, Dar es Salaam, Tanzania
Tel: +255-51-30610
Fax: +255-51-113260

M. Mangani

Department of Veterinary Services
P.O. Box 50060, Lusaka, Zambia
Tel: +260-1-252608
Fax: +260-1-252608/236283
Email: cvri@zamnet.zm

S. Mbarubukeye

National Veterinary Laboratory
P.O. Box 804, Kigali, Rwanda
Tel: +250-8-7013
Fax: +250-8-3689

S. Mbogo

NVRC-Muguga
P.O. Box 32, Kikuyu, Kenya
Tel: +254-154-33412
Fax: +254-154-32450
Email: skmbogo@africaonline.co.ke

N. McHardy

NVRI-Muguga
P.O. Box 32, Kikuyu, Kenya
Tel: +254-154-33412
Fax: +254-154-884331

D. McKeever

ILRI
P.O. Box 30709, Nairobi, Kenya
Tel: +254-2-630743
Fax: +254-2-631499
Email: d.mckeever@cgiar.org

P. McLeod

KARI
P.O. Box 30465, Nairobi, Kenya
Tel: +254-2-580637
Fax: +254-2-583344
Email: hyperlink mailto:mcLeod@arcc.or.ke mcLeod@arcc.or.ke

J. Melewas

Livestock Development
P.O. Box 9152, Dar es Salaam, Tanzania
Tel: +255-51-30610
Fax: +255-51-113260

M. Moran

FAO Representative Office
P.O. Box 521, Kampala, Uganda
Tel: +256-42-20431
Fax: +256-41-250579

S. Morzaria

ILRI
P.O. Box 30709, Nairobi, Kenya
Tel: +254-2-630743
Fax: +254-2-631499
Email: s.morzaria@cgiar.org

A. Mukhebi

ILRI
P.O. Box 30709, Nairobi, Kenya
Tel: +254-2-630743
Fax: +254-2-631499
Email: a.mukhebi@cgnet.com

G. Muraguri

NVRC–Muguga
P.O. Box 32, Kikuyu, Kenya
Tel: +254–154–33450
Fax: +254–154–32450

J. Musiime

OAU/IBAR
P.O. Box 30786, Nairobi, Kenya
Tel: +254–2–338544
Fax: +254–2–332046/220546
Email: parcepid@africaonline.co.ke

F. Musisi

FAO
FAO Office,
P.O. Box 30750, Lilongwe 3, Malawi
Tel: +265–734093
Fax: +265–743781

A. Musoke

ILRI
P.O. Box 30709, Nairobi, Kenya
Tel: +254–2–630743
Fax: +254–2–631499
Email: a.musoke@cgnnet.com

J. Mutugi

FAO Representative Office
P.O. Box 30750, Lilongwe 3, Malawi
Tel: +265–734093
Fax: +265–743781

R. Nsubunga-Mutaka

LIRI (NARO)
P.O. Box 24, Entebbe, Uganda
Tel: +256–41–320431
Fax: +256–41–349422

P. Ociba

Department of Veterinary Services
P.O. Box 513, Kampala, Uganda
Tel: +256–41–320627
Fax: +256–41–320237
Email: parug@imul.com

R. Payne

NVRC–Muguga
P.O. Box 32, Kikuyu, Kenya
Tel: +254–154–32703
Fax: +254–154–884361

E. Peeler

c/o UC, Box 30465, Nairobi, Kenya
Tel: +254–2–882301
Fax: +254–2–884016
Email: ejpeeler@users.afrinie.co.re

R. Rumberia

NVRC–Muguga
P.O. Box 32, Kikuyu, Kenya
Tel: +254–154–32106/7
Fax: +254–154–884361

P. Schmidt

DANIDA
Ministry of Foreign Affairs
Asiatisk Plads 2
1448 Copenhagen
Denmark
Tel: +45–51–3392000

P. Spooner

ILRI
P.O. Box 30709, Nairobi, Kenya
Tel: +254–2–630743
Fax: +254–2–631499
Email: p.spooner@cgiar.org

R. Thwala

Veterinary Services
P.O. Box 162, Mbabane, Swaziland
Tel: +268–42731/5
Fax: +268–44700

U. Ushewokunze-Obatolu

Veterinary Research Laboratory
P.O. Box CY551
Causeway, Harare, Zimbabwe
Tel: +263–4–705885
Fax: +263–4–707952
Email: ncube@healthnet.zw

J. Wafula

KARI Headquarters
P.O. Box 57811, Nairobi, Kenya
Tel: +254–2–583343
Fax: +254–2–583343
Email: jswafula@arcc.or.ke